ANTIPLATELET AGENTS IN STROKE PREVENTION

EDITED BY: Gergely Feher, David Hargroves, Zsolt Illes, Peter Klivenyi,
Liping Liu and Laszlo Szapary

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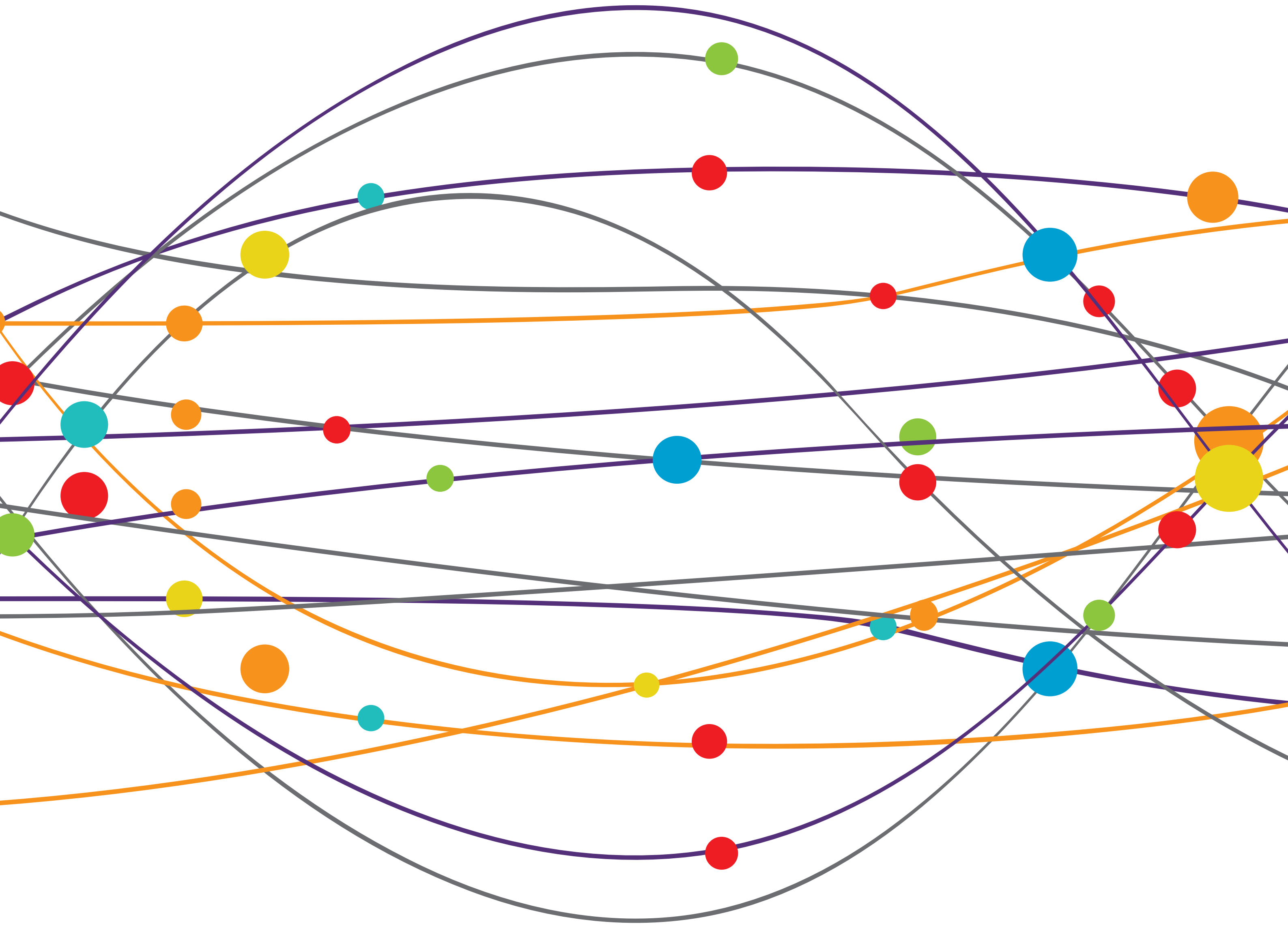

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ANTIPLATELET AGENTS IN STROKE PREVENTION

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Editorial: Antiplatelet Agents in Stroke Prevention

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Keywords: stroke, antiplatelet agent, resistance, biomarker, outcome

Editorial on the Research Topic

Antiplatelet Agents in Stroke Prevention

Stroke is the leading cause of disability and the second most common cause of death worldwide based on the results of the Global Burden of Diseases Study (1). More than 80% of all stroke syndromes are ischemic infarcts and their prevalence and cost will undoubtedly rise as aging populations increase (2). Despite extensive risk factor stratification and enhanced brain imaging, the etiology of stroke is still unknown in a significant proportion of patients. However, atherosclerosis, which is a low-grade inflammatory condition with detectable biomarkers, is the most likely culprit in most strokes (3).

Platelets play an essential role in the pathogenesis of atherothrombotic cardio- and cerebrovascular events, thus justifying the use of antiplatelet agents in their prevention. In their mini review, Valis and his workgroup summarized the evidence-based role of antiplatelet agents in the secondary prevention of non-cardioembolic stroke including aspirin, clopidogrel, dual antiplatelet therapy, and alternative agents such as cilostazol and ticagrelor Vališ et al.

Despite their efficacy, patients on these medications continue to suffer complications, which raises the possibility of the so-called "antiplatelet resistance" that is used to refer to the inability to protect individuals from thrombotic events (4). Due to the lack of standard methodology and randomized trials involving cerebrovascular patients, the clinical significance of antiplatelet resistance is contradictory (5). However, observational studies have shown an increased rate of ischemic cerebrovascular events in patients with high on-treatment of platelet reactivity (HPR) (so called resistance) in patients with both single (SAPT) and dual antiplatelet therapy (DAPT) (6).

Kang et al. analyzed the risk factors of clopidogrel resistance in patients taking mono- and dual therapy Kang et al. They demonstrated that HPR is more frequent in recurrent stroke patients receiving clopidogrel SAPT than in those receiving DAPT, and its risk factors may differ. The rates of HPR and clopidogrel resistance were lower in current smokers, which is rather surprising as smoking is one of the most important risk factors of atherosclerotic diseases. The role of smokers' paradox is not well-understood and merits further investigation.

In their paper Schrick et al. presented a modified platelet function test (mPFT) wherein they not only tested whole blood (WB), but also analyzed 1-h gravity sedimentation of the separated upper (UB) and lower half blood (LB) samples using Multiplate Analyzer to detect HPR as well as neutrophil antisedimentation rate (NAR) Shrick et al. This pilot study suggested that upward motion of platelets might be associated with increased thrombotic tendency.

It is worth noting that assessment of response to aspirin, GPI-s, or PAR-inhibitors is clinically not established as suggested by the Working Group on Thrombosis of the European Society of Cardiology (7). The most reliable, clinically best validated, and most widely used assays measured

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the effect of P2Y12-inhibitors (clopidogrel or prasugrel) and the recommended techniques were VASP-P assay, the VerifyNow device, and the Multiplate analyzer (7, 8). However, the routine use of platelet function testing is still not recommended (7, 8).

HPR can be associated with more ischemic events and recent studies have shown an increased bleeding risk in patients with low platelet reactivity (LPR) (9). Rosafio et al. also presented an interesting clinical case scenario of an aspirin ultra-responder patient Rosafio et al.

Since the coagulation system plays an important role in stroke pathogenesis, blood biomarkers of coagulation, and inflammation might render the possibility to differentiate which patients are at risk of poor clinical outcome. The ability to predict clinical outcome after an ischemic stroke may help to improve the selection of the most appropriate therapy (10). Based on recent studies, hemostatic changes during acute stroke in relation to antiplatelet resistance may predict the severity of an ischemic stroke.

In their in-depth review, Alhazzani et al. summarized the integration of specific biomarkers, genotype-, as well as phenotype-related data in antiplatelet therapy stratification in patients with acute ischemic stroke, which could be of great clinical impact on outcome Alhazzani et al.

Platelet endothelial aggregation receptor-1 (PEAR1) rs12041331 has been reported to affect agonist-stimulated platelet aggregation which can be associated with HPR in aspirin and clopidogrel treated patients, increasing the risk of unfavorable outcome. An observational Chinese study conducted by Zhang et al. could not confirm its role either in ischemic nor in bleeding events in TIA or minor stroke patients taking DAPT, doubting its prognostic value Zhang et al.

There is no doubt that taking antiplatelet agents or anticoagulants increases the risk of bleeding complications. Antiplatelet (especially DAPT) pretreatment potentially increases the risk of intracranial bleeding in thrombolyis/thrombectomy situations as well as in patients with traumatic brain injuries (11, 12). The potential harmful effects of DAPT have also been confirmed in this issue by the research of Lin et al. in more than 1,000 elderly patients with moderate to severe strokes who underwent systemic thrombolysis Lin et al. Although the patient cohorts were quite homogenous, the DAPT group contained relatively few patients (∼2% of the study cohort). Finally, based on a recent meta-analysis consisting of more than 60,000 patients, DAPT did not appear to be associated with a higher risk of adverse outcomes in thrombolyzed stroke patients, so dual pre-treatment is not an indication to withdraw treatment, which is also confirmed by the authors (13).

Single small subcortical infarction (SSSI or lacunar stroke) accounts for 25% of all strokes and has heterogenous pathogenesis. Recent studies have shown an increased bleeding risk of SSSI patients, especially for those with underlying small vessel disease or taking DAPT (14). As the optimal treatment of these patients is not entirely clarified, Wang et al. analyzed the data of the CHANCE trial dividing patients into different subgroups based on antiplatelet treatment and SSSI etiology Wang et al. They could not find any differences in the outcome of different subgroups, which merits further investigation.

Endovascular treatments have recently proven to be effective in improving functional outcomes for selected patients with large vessel occlusion, although it can cause injury to endothelial cells leading to activation of local platelet aggregation and subsequent early reocclusion, and therefore more effective and safe thrombolytic agents are required (15). Glycoprotein (GP) IIb-IIIa inhibitors are short-acting selective reversible antiplatelet agents widely used in acute coronary syndromes and have recently emerged as promising therapeutic agents for ischemic stroke management. Among them, tirofiban may be considered safe in low doses (15). Two studies focused on the efficacy and safety of tirofiban in relation to the management of large vessel occlusion (LVO) including thrombectomy. Huo et al. showed its beneficial effects in 650 ischemic stroke patients; based on their findings tirofiban was found to be associated with superior clinical outcomes in anterior circulation stroke and major stroke patients and had a trend to lower the risk of mortality at 90-day follow-ups with no increase in bleeding rates compared to the non-tirofiban group Huo et al. In the other study presented by Ma et al. covering ∼200 patients, no significant differences in safety and efficacy outcomes on successful recanalization, clinical improvement, or 3-month mRS could be found between the tirofiban and non-tirofiban groups Ma et al. The administration of tirofiban seems to be safe in LVO patients but its efficacy and safety merits further investigation.

Intracerebral hemorrhage (ICH) may be caused by antiplatelet treatment and prior treatment may be associated with worse clinical outcomes; however, previous studies on ICH growth and outcome have found conflicting results (16, 17). In their meta-analysis of 31 studies, Wu et al. found no association with hematoma expansion or functional outcomes in ICH patients, but increased mortality rates raised the possibility of the introduction of early-time platelet function reversal strategies Wu et al. It is worth noting that the randomized PATCH trial found platelet transfusion to be inferior compared to standard care in ICH patients (18).

The rupture of an intracranial aneurysm could be a life-threatening disease accounting for a relatively small but significant number of stroke syndromes. The role of prior antiplatelet use on the risk of bleeding and outcome is not well-studied. In their interesting meta-analysis covering nearly 9,000 participants, Yang et al. found that prior aspirin use was associated with a significantly lower risk of aneurysm growth and rupture, suggesting the potential protective effect of aspirin Yang et al. However, it is not well-understood and merits further investigations.

AUTHOR CONTRIBUTIONS

This editorial was written by GF and checked by DH, ZI, PK, LL, and LS. All authors contributed to the article and approved the submitted version.

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Safety and Efficacy of Tirofiban for Acute Ischemic Stroke Patients With Large Artery Atherosclerosis Stroke Etiology Undergoing Endovascular Therapy

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Objective: To investigate the safety and efficacy of tirofiban in acute ischemic stroke (AIS) patients with large artery atherosclerosis (LAA) stroke etiology receiving endovascular therapy (EVT).

Methods: In this multi-center prospective study, patients who were considered to have an indication received a low dose intra-arterial bolus (0.25–1 mg) of tirofiban. The safety and efficacy outcomes at 90-day follow-ups included symptomatic intracranial hemorrhage (sICH), recanalization rate, functional outcome, and mortality.

Results: Among the 649 AIS patients with LAA, those in the tirofiban group (n = 244) showed higher systolic blood pressure (BP) and NIHSS score on admission, puncture-to-recanalization time, lower frequency of intravenous thrombolysis and intra-arterial thrombolysis, higher frequency of antiplatelet, heparinization, mechanical stent retrieval, aspiration, balloon angioplasty, and more retrieval times compared with those in the non-tirofiban group (n = 405) (all $P < 0.05$). Tirofiban was found to be associated with superior clinical outcomes in anterior circulation stroke and major stroke patients [adjusted odds ratio (OR) = 2.163, 95% confidence interval (CI) = 1.130–4.140, $P = 0.02$ and adjusted OR = 2.361, 95% CI = 1.326–4.202, $P = 0.004$, respectively] and a lower risk of mortality at 90-day follow-ups (adjusted OR = 0.159, 95% CI = 0.042–0.599, $P = 0.007$ and adjusted OR = 0.252, 95% CI = 0.103–0.621, $P = 0.003$, respectively). There was no significant difference in sICH between the two groups.

Conclusions: Tirofiban in AIS patients with LAA undergoing EVT is safe and may benefit the functional outcomes in anterior circulation and major stroke patients and showed a trend for reduced mortality.

Keywords: tirofiban, endovascular therapy, acute ischemic stroke, large artery atherosclerosis, safety and efficacy, clinical outcome

INTRODUCTION

The non-peptide platelet GP IIb/IIIa receptor inhibitor, tirofiban, has been increasingly applied as a rescue therapy, by either intra-arterial or intravenous route during endovascular treatment (EVT) (1–8). Tirofiban can selectively and efficiently block the final pathway of platelet aggregation and subsequent thrombus formation in atherosclerotic lesions (9, 10). Recent metaanalysis studies have reported that the safety profile and efficacy of tirofiban may make it a potential choice for treatment in patients with acute ischemic stroke (AIS) (11–14). It has also been reported to be more feasible and effective in AIS patients with large artery atherosclerosis (LAA) compared to those with cardioembolic stroke etiology (15, 16). However, the treatment results were inconsistent (1, 17, 18) and a study reported an increased risk of symptomatic intracranial hemorrhage (sICH) and a poor outcome in patients treated with tirofiban during mechanical thrombectomy (19). Moreover, to the best of our knowledge, there are no reports on which stratified population may benefit the most from rescue tirofiban therapy.

To address this issue, we explored the safety and efficacy of rescue tirofiban treatment in AIS patients with LAA stroke etiology and evaluated which stratified population gained the most benefit from rescue tirofiban in a large multi-center cohort study in China.

METHODS

Patient Selection and Data Collection

This multi-center nationwide prospective study of an Acute Ischemic Stroke Cooperation group in the Endovascular Treatment (ANGEL) registry recruited 917 Chinese patients with AIS to evaluate EVT delivery and improve EVT. The study protocol was similar to our previous research (20). The present study was approved by the ethics committee at each participating center, and informed consent was obtained from all participants prior to commencing the study.

Patient's baseline data, such as age, gender, systolic blood pressure (SBP), the National Institutes of Health Stroke Scale (NIHSS) score, Alberta Stroke Program Early CT Score (ASPECTS), time intervals [onset-to-door (OTD), door-to-puncture (DTP), puncture-to-recanalization (PTR), onset-to-puncture (OTP), and onset-to-recanalization (OTR)], were recorded within 24 h after admission. Vascular risk factors included atrial fibrillation, diabetes mellitus, history of previous stroke, hypertension, smoking, and drinking. The data related to the peri-procedural anti-thrombotic and anticoagulation therapies, such as administration of antiplatelets, bridging intravenous thrombolysis (IVT), and heparin, were recorded as along with the procedural techniques.

AIS patients undergoing EVT were divided into tirofiban and non-tirofiban groups. All EVT procedures were performed by neurointerventionalists with extensive experience in neurovascular intervention.

Dose and Indication of Rescue Tirofiban

Rescue tirofiban with low-dose intra-arterial bolus (0.25–1 mg) is suggested when there are the following indications: (1) severe residual stenosis or instant re-occlusion requiring emergency stenting or balloon angioplasty; (2) stent retrieval times > 3 passes for presumed vascular endothelial injury or instant re-occlusion; and (3) severe degree of *in situ* atherosclerosis with a tendency to early re-occlusion. Low dose rescue tirofiban followed by intravenous continuous infusion (0.1 μg/kg/min) for 12–24 h is suggested when there is no indication of post-operative intracranial hemorrhage following a CT examination.

Clinical Efficacy and Safety Outcomes

SICH, which was defined by the European Cooperative Acute

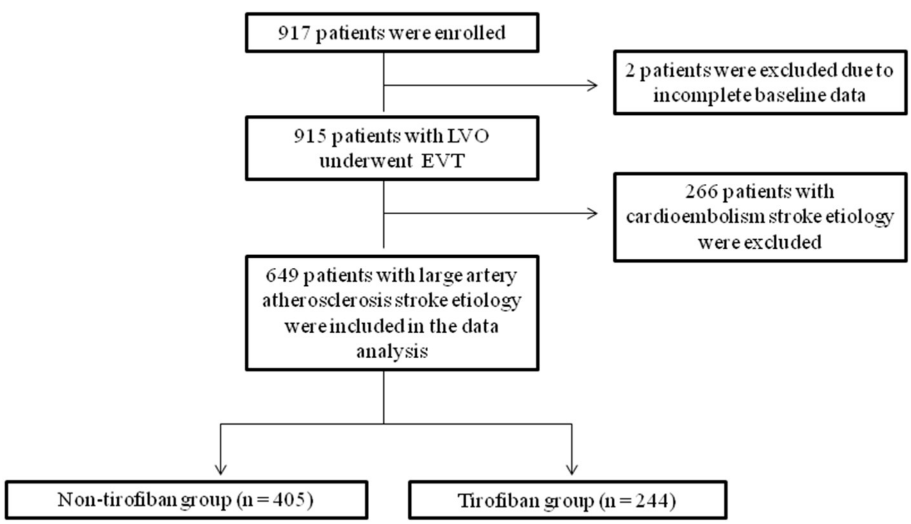

FIGURE 1 | Flow chart showing patient selection.

Stroke Study III (ECASS-III) trial as evidence of hemorrhage on a CT or MRI, was considered a primary safety endpoint. The primary efficacy endpoints were the functional independence (mRS 0-2) and mortality at 90 day follow-ups. A successful recanalization, which was defined as modified Thrombolysis in Cerebral Infarction (mTICI), is considered the secondary efficacy endpoint in the present study.

Statistical Analysis

The baseline characteristics of patients were compared between the tirofiban and non-tirofiban groups. The χ^2 test or Kruskal-Wallis test was used to compare the baseline characteristics and safety and efficacy outcomes at 90 days between the tirofiban and non-tirofiban groups. The logistic regression model was used to evaluate the odds ratios (OR)/hazard ratio (HR) with a 95%

TABLE 1 | Patient's Baseline and procedural characteristics.

Variables	Total (n = 649)	Non-tirofiban (n = 405)	Tirofiban (n = 244)	*P*-value
Age, mean ± SD	63 (55–71)	63 (54–71)	64 (55–70.75)	0.784
Male	464 (71.5)	282 (69.6)	182 (74.6)	0.175
SBP, mean + SD	148 (133–162)	147 (130–160)	150 (138–168.75)	**0.037**
Admission NIHSS, median (IQR)	13 (8–18)	13 (7–17.5)	14 (10–20.75)	**0.005**
ASPECTS (AC only)	8 (7–8)	8 (7–8)	8 (7–8)	0.959
Vascular risk factors				
Atrial Fibrillation	55 (8.5)	43 (10.6)	12 (4.9)	**0.012**
Diabetes Mellitus	107 (16.5)	65 (16)	42 (17.2)	0.699
Previous stroke	70 (10.8)	40 (9.9)	30 (12.3)	0.336
Hypertension	376 (57.9)	224 (55.3)	152 (62.3)	0.081
Smoking	238 (36.7)	132 (32.6)	106 (43.4)	**0.005**
Drinking	111 (17.1)	65 (16)	46 (18.9)	0.358
Anterior circulation	488 (75.2)	324 (80)	164 (67.2)	**<0.001**
Posterior circulation	161 (24.8)	81 (20)	80 (32.8)	**<0.001**
Occlusion sites				
ICA	209 (32.2)	137 (33.8)	72 (29.5)	0.254
M1	225 (34.7)	147 (36.3)	78 (32)	0.262
M2/3	50 (7.7)	38 (9.4)	12 (4.9)	**0.039**
ACA	4 (0.6)	2 (0.5)	2 (0.8)	1
VA	78 (12)	39 (9.6)	39 (16)	**0.016**
BA	68 (10.5)	28 (6.9)	40 (16.4)	**<0.001**
PCA	15 (2.3)	14 (3.5)	1 (0.4)	**0.026**
OTD time, median (IQR), min	180 (110–300)	180 (102–300)	203.5 (120–313.5)	0.076
DTP time, median (IQR), min	116 (74–167.5)	119(80–168.5)	110 (62.25–167.25)	0.123
PTR time, median (IQR), min	80 (55–115)	119 (80–168.5)	110 (62.25–167.25)	0.095
OTP time, median (IQR), min	330 (225–463.5)	320 (223.5–453.5)	340 (233.5–490)	0.239
OTR time, median (IQR), min	420 (320–576)	410 (316.25–550)	438.5 (323.75–629.25)	0.103
Antitrombotic and anticoagulation				
Antiplatelet	148 (22.8)	55 (13.6)	93 (38.1)	**<0.001**
Bridging IVT	174 (26.8)	125 (30.9)	49 (20.1)	**0.003**
Heparin during EVT	242 (37.3)	141 (34.8)	101 (41.4)	0.093
Procedural characteristics				
General anesthesia	217 (33.4)	106 (26.2)	111 (45.5)	**<0.001**
Stent retrieval	428 (65.9)	242 (59.8)	186 (76.2)	**<0.001**
Aspiration	36 (5.5)	11 (2.7)	25 (10.2)	**<0.001**
Intra-arterial thrombolysis	152 (23.4)	124 (30.6)	28 (11.5)	**<0.001**
Balloon angioplasty	85 (13.1)	42 (10.4)	43 (17.6)	**<0.001**
Stent angioplasty	126 (19.4)	70 (17.3)	56 (23)	0.077
retrieval times, median (IQR)	1 (0–1)	1 (0–1)	1 (1–1)	**0.002**

SD, standard deviation; SBP, systolic blood pressure; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale score; ASPECTS, Alberta Stroke Program Early CT score; ICA, internal carotid artery; M1, middle cerebral artery M1 segment; M2/3, middle cerebral artery M2/3 segment; ACA, anterior cerebral artery; OTD, onset-to-door; DTP, door-to-puncture; PTR, puncture-to-recanalization; OTP, onset-to-puncture; OTR, onset-to-recanalization; IVT, intravenous thrombolysis; EVT, endovascular treatment. Bold values indicates statistical significance.

confidence interval (CI) of safety and efficacy endpoints (sICH), mTICI grade 2b-3, complete reperfusion (mTICI 3), functional independence (mRS 0-2), and mortality with or without use of tirofiban. The multivariate models were adjusted for some potential confounders with *P < 0.05* in univariate analysis, which included SBP, NIHSS, atrial fibrillation, smoking history, anterior and posterior circulation, occlusion of the M2 or M3 segment of the middle cerebral artery (MCA) M2/3 segment, vertebral artery (VA), basilar artery (BA), posterior cerebral artery (PCA), antiplatelet, bridging IVT during EVT, general anesthesia, MT stent retrieval and aspiration, balloon angioplasty and intra-arterial thrombolysis, and retrieval times. A *P-value < 0.05* was considered statistically significant. All statistical analyses were conducted using SPSS 20.0 software (IBM, Armonk, NY, USA).

RESULTS

Baseline Characteristics of Patients

Two of the 917 patients were excluded from the data analysis due to missing baseline data. Subsequently, 266 patients with embolic

TABLE 2 | Safety and efficacy outcomes grouped by tirofiban in LAA patients.

Variables	Total (*n* = 649)	Non-tirofiban (*n* = 405)	Tirofiban (*n* = 244)	OR/HR	*P*-value	adjusted OR/HR	*P*-value
Safety outcome							
sICH	27 (4.2)	16 (4)	11 (4.5)	1.148 (0.524–2.516)	0.731	0.998 (0.021–46.825	0.999
Recanalization							
mTICI 2b/3	605 (93.2)	377 (93.1)	228 (93.4)	1.058 (0.56–1.999)	0.861	0.308 (0.104–0.911)	**0.033**
Functional outcome at 90-days							
mRS 0–1	295 (45.5)	182 (44.9)	113 (46.3)	1.057 (0.768–1.454)	0.734	1.819 (1.064–3.110)	**0.029**
mRS 0–2	364 (56.1)	227 (56)	137 (56.1)	1.004 (0.729–1.383)	0.981	1.849 (1.065–3.212)	**0.029**
mRS 6	87 (13.4)	59 (14.6)	28 (11.5)	0.76 (0.470–1.230)	0.264	0.2 (0.079–0.507)	**0.001**

sICH, symptomatic intracranial hemorrhage; aICH, asymptomatic intracranial hemorrhage; mTICI, modified treatment in cerebral infarction; mRS, modified rankin score; OR, odds ratio; HR, hazard ratio.

adjusted for SBP, NIHSS, atrial fibrillation, smoking, anterior circulation, posterior circulation, MCA M23 segment, VA, BA, PCA, antiplatelet, Intravenous thrombolysis, general anesthesia, MT stent retrieval, MT aspiration, intra-arterial thrombolysis, balloon angioplasty and retrieval times. Bold values indicates statistical significance.

TABLE 3 | Safety and efficacy outcomes grouped by tirofiban in LAA patients stratified according to anterior and posterior circulation stroke.

Anterior Circulation							
Variables	**Total (*n* = 488)**	**Non-tirofiban (*n* = 324)**	**Tirofiban (*n* = 164)**	**OR/HR**	***P*-value**	**adjusted OR/HR**	***P*-value**
Safety outcome							
sICH	22 (4.5)	14 (4.3)	8 (4.9)	1.136 (0.466–2.764)	0.779	3.52×10^{10} (0)	0.997
Recanalization							
mTICI 2b/3	456 (93.4)	302 (93.2)	154 (93.9)	1.122 (0.518–2.428)	0.77	0.343 (0.053–2.200)	0.259
Functional outcome at 90-days							
mRS 0-1	216 (44.3)	135 (41.7)	81 (49.4)	1.366 (0.937–1.993)	0.105	2.163 (1.130–4.140)	**0.02**
mRS 0-2	272 (55.7)	174 (53.7)	98 (59.8)	1.28 (0.875–1.873)	0.204	1.845 (0.946–3.598)	0.072
mRS 6	53 (10.9)	40 (12.3)	13 (7.9)	0.611 (0.317–1.178)	0.141	0.159 (0.042–0.599)	**0.007**
Posterior Circulation							
Variables	**Total (*n* = 161)**	**Non-tirofiban (*n* = 81)**	**Tirofiban (*n* = 80)**	**OR/HR**	***P*-value**	**adjusted OR/HR**	***P*-value**
Safety outcome							
sICH	5 (3.1)	2 (2.5)	3 (3.8)	1.539 (0.250–9.465)	0.642	2.27×10^{20} (0)	0.993
Recanalization							
mTICI 2b/3	149 (92.5)	75 (92.6)	74 (92.5)	0.987 (0.304–3.199)	0.982	0.379 (0.047–3.066)	0.363
Functional outcome at 90-days							
mRS 0-1	79 (49.1)	47 (58)	32 (40)	0.482 (0.257–0.904)	0.023	2.566 (0.597–11.031	0.205
mRS 0-2	92 (57.1)	53 (65.4)	39 (48.8)	0.503 (0.267–0.947)	0.033	4.547 (0.714–28.942)	0.109
mRS 6	34 (21.1)	19 (23.5)	15 (18.8)	0.753 (0.352–1.612)	0.465	0.001 (0.000–0.188)	**0.009**

sICH, symptomatic intracranial hemorrhage; aICH, asymptomatic intracranial hemorrhage; mTICI, modified treatment in cerebral infarction; mRS, modified rankin score; OR, odds ratio; HR, hazard ratio.

adjusted for SBP, NIHSS, atrial fibrillation, smoking, MCA M23 segment, VA, BA, PCA, antiplatelet, Intravenous thrombolysis, general anesthesia, MT stent retrieval, MT aspiration, intra-arterial thrombolysis, balloon angioplasty and retrieval times. Bold values indicates statistical significance.

TABLE 4 | Safety and efficacy outcomes grouped by tirofiban in LAA patients stratified according to minor (NIHSS 0–5)and major (NIHSS > 5) stroke.

Minor (NIHSS 0–5) stroke							
Variables	**Total ($n = 113$)**	**Non-tirofiban ($n = 75$)**	**Tirofiban ($n = 38$)**	**OR/HR**	***P*-value**	**adjusted OR/HR**	***P*-value**
Safety outcome							
sICH	2 (1.8)	1 (1.3)	1 (2.6)	2 (0.122–32.881)	0.628	0 (0)	0.993
Recanalization							
mTICI 2b/3	103 (91.2)	70 (93.3)	33 (86.8)	0.471 (0.128–1.742)	0.259	0.095 (0.008–1.070)	0.057
Functional outcome at 90-days							
mRS 0-1	87 (77)	63 (84)	24 (63.2)	0.327 (0.132–0.806)	**0.015**	0.466 (0.122–1.785)	0.265
mRS 0-2	99 (87.6)	69 (92)	30 (78.9)	0.326 (0.104–1.022)	**0.054**	0.551 (0.1–3.034)	0.494
mRS 6	4 (3.5)	2 (2.7)	2 (5.3)	2.028 (0.274–14.986)	0.488	7.76×10^3(0)	0.999
Major (NIHSS > 5) stroke							
Variables	**Total ($n = 536$)**	**Non-tirofiban ($n = 330$)**	**Tirofiban ($n = 206$)**	**OR/HR**	***P*-value**	**adjusted OR/HR**	***P*-value**
Safety outcome							
sICH	25 (4.7)	15 (4.5)	10 (4.9)	1.071 (0.472–2.432)	0.869	0.569 (0.071–4.584)	0.596
Recanalization							
mTICI 2b/3	502 (93.7)	307 (93)	195 (94.7)	1.328 (0.633–2.785)	0.453	0.784 (0.183–23.360)	0.743
Functional outcome at 90-days							
mRS 0-1	208 (38.8)	119 (36.1)	89 (43.2)	1.349 (0.945–1.925)	0.099	2.361 (1.326–4.202)	**0.004**
mRS 0-2	265 (49.4)	158 (47.9)	107 (51.9)	1.177 (0.83–1.667)	0.36	1.944 (1.090–3.469)	**0.024**
mRS 6	83 (15.5)	57 (17.3)	26 (12.6)	0.692 (0.419–1.141	0.149	0.252 (0.103–0.621)	**0.003**

sICH, symptomatic intracranial hemorrhage; aICH, asymptomatic intracranial hemorrhage; mTICI, modified treatment in cerebral infarction; mRS, modified rankin score; OR, odds ratio; HR, hazard ratio.

adjusted for SBP, NIHSS, atrial fibrillation, smoking, anterior circulation, posterior circulation, MCA M23 segment, VA, BA, PCA, antiplatelet, Intravenous thrombolysis, general anesthesia, MT stent retrieval, MT aspiration, intra-arterial thrombolysis, balloon angioplasty and retrieval times. Bold values indicates statistical significance.

stroke etiology were also excluded. Finally, 649 patients with large vessel atherosclerosis who underwent EVT with or without receiving tirofiban were analyzed (**Figure 1**).

As shown in **Table 1**, the median age of patients was 63 (55–71) years, 464 (71.5%) patients were male, and 244 (37.6%) had received tirofiban. In the tirofiban group, SBP and NIHSS on admission were relatively higher and smoking history was more frequent, while atrial fibrillation was less obvious than those in the non-tirofiban group (all $P < 0.05$). Rescue tirofiban was used more in the posterior circulation (particularly VA, BA, and PCA), but less in the anterior circulation group (particularly MCI M23 segment). In the tirofiban group, general anesthesia, stent retrieval, MT aspiration, and balloon angioplasty were more frequently performed as compared to the non-tirofiban group (45.5 vs. 26.2%, $P < 0.001$), (76.2 vs. 59.8%), $P < 0.001$), (10.2 vs. 2.7%), $P < 0.001$), and (17.6 vs. 10.4%), $P = 0.008$)), respectively. Moreover, anti-platelet therapy was administered more in the tirofiban group (38.1 vs. 13.6%), $P < 0.001$). Meanwhile, the proportions of bridging IVT and intra-arterial thrombolysis were less in the tirofiban group compared to the non-tirofiban group (20.1 vs. 30.9%, $P = 0.003$) and (11.5 vs. 30.6%, $P < 0.001$).

There was no significant difference in age, gender, other vascular risk factors (diabetes mellitus, previous stroke, hypertension, and drinking), other occlusion sites (ICA, MCA M1, or ACA), time workflow (OTD, DTP, PTR, OTP, and OTR), heparinization during EVT, and stent angioplasty between the tirofiban and non-tirofiban groups (all $P > 0.05$).

Safety and Efficacy Outcomes

The safety and efficacy outcomes are shown in **Tables 2**, **3**, **4**. Overall, 27 (4.2%) patients developed sICH within 24 h post-EVT, and no significant difference was noted in the sICH incidence between the tirofiban group and the non-tirofiban group ($P > 0.05$). Tirofiban was not correlated with the incidence of sICH (adjusted HR 0.998; 95% CI 0.021–46.825; $P = 0.999$) even after adjusting for some potential confounders. Similar results were demonstrated when the population was stratified into anterior/posterior circulation and minor (NIHSS 0–5)/major (NIHSS > 5) stroke (all $P > 0.05$).

At 90 day follow-ups, excellent outcome (mRS0-1) and functional independence (mRS0-2) could be achieved in 295 (45.5%) and 182 (44.9%) patients, respectively. However, 87 (13.4%) patients had died (mRS 6) by the three-month follow-up (**Table 2**, **Figure 2**). A slightly higher rate of superior clinical outcomes and a lower risk of mortality were found in patients who received tirofiban. Moreover, tirofiban was associated with excellent outcomes and functional independence after adjusting for several potential confounders (adjusted OR,

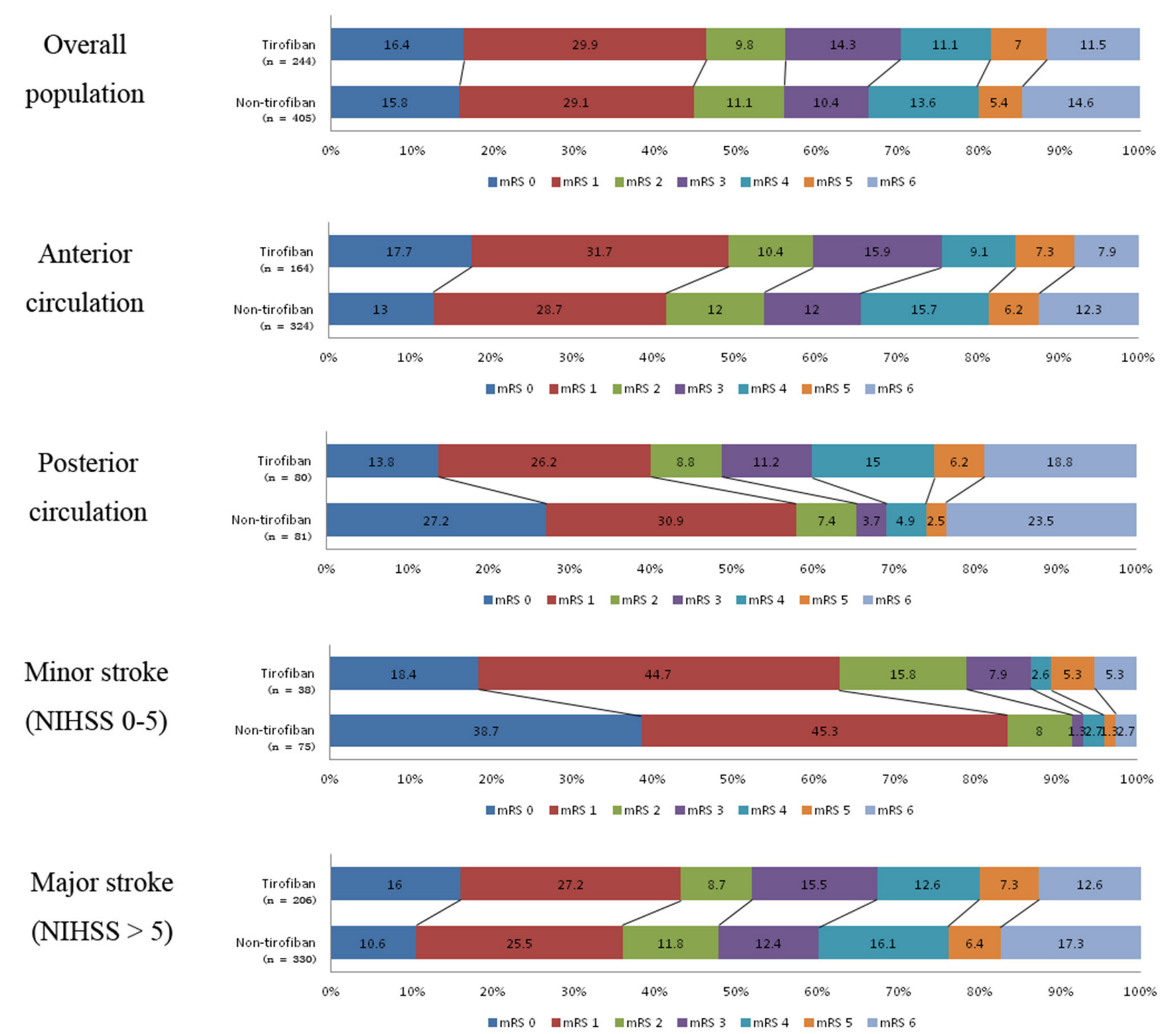

FIGURE 2 | Distribution of mRS scores at 3-month follow-up between tirofiban and non-tirofiban in different stratification.

1.819; 95%CI, 1.064–3.110; $P = 0.029$ and OR, 1.849; 95%CI, 1.065–3.212; $P = 0.029$, respectively). Further analysis showed a strong association of tirofiban with favorable functional outcomes in the anterior circulation (adjusted OR 2.163; 95%CI, 1.130–4.140; $P = 0.02$) and NIHSS > 5 (adjusted OR 2.361; 95% CI, 1.326–4.202; $P = 0.004$). Furthermore, tirofiban was significantly correlated with a lower risk of mortality (adjusted HR 0.2; 95% CI, 0.079–0.507; $P = 0.001$) even after adjusting for potential factors. This strong association was significantly demonstrated in the anterior circulation (adjusted OR 0.159; 95% CI, 0.042–0.599; $P = 0.007$), posterior circulation (adjusted OR 0.001; 95% CI, 0.000–0.188; $P = 0.009$), and NIHSS > 5 (adjusted OR 0.252; 95% CI, 0.103–0.621; $P = 0.003$).

DISCUSSION

The present study showed that rescue tirofiban offers a safe outcome for the risk of sICH in AIS patients with LAS who received EVT. In the LAS population, rescue tirofiban showed superior clinical outcomes in patients with an AC stroke and NIHSS > 5. Rescue tirofiban may lower the mortality risk in these stratified patients as well as those with a PC stroke.

The clinical benefit of tirofiban remains controversial in AIS patients who received recanalization therapy. Previous studies have reported the feasibility and effectiveness of tirofiban, and suggested tirofiban use in failed mechanical thrombectomy (15–17). In contrast, another study reported no clinical benefit and also highlighted safety concerns of tirofiban (19). These conflicting results might be attributed to the small sample size, various treatment strategies, and uncontrolled study design in these preliminary studies. Thus, special caution is needed when interpreting these results. However, the majority of these studies shared similar indications that tirofiban is more beneficial for LAA patients. Moreover, a recent meta-analysis indicated that tirofiban use is safe and appears to be effective in treating AIS patients (11–14). Since we compared tirofiban and non-tirofiban use only in patients with LAA, the clinical benefit of rescue tirofiban was more significant in this study. Interestingly, our results demonstrated that patients with an AC stroke and a major stroke received more clinical benefit either through functional outcome or mortality risk from rescue tirofiban, while no significant clinical benefit was found in patients with PC stroke and minor stroke. Despite no functional benefit in those with a PC stroke, rescue tirofiban was advantageous in lowering the mortality rate in the study.

This study was in agreement with previous findings that showed rescue tirofiban did not affect recanalization (1, 21). However, the clinical benefit of rescue tirofiban in LAA patients is that it prevents subsequent ischemic events and the mechanisms have been well-described. Tirofiban has anti-inflammatory effects and may stabilize inflamed stenotic lesions and maintain blood flow, which is helpful in preventing ischemic events caused by inflammation and platelet aggregation (22). In addition, this rescue therapy might benefit cases with stent retrieval times > 3, which are prone to vascular endothelial injury or instant re-occlusion (21, 23). Moreover, it is recommended to use tirofiban in patients with no history of anti-platelet, as it has more significant dose-dependent blockade effects on platelet aggregation and thrombosis (24, 25). Tirofiban is a highly selective platelet antagonist that can block fibrinogen, and its mechanical effect is usually maintained for 20 min after administration (26).

The current study showed that not all LAA patients may receive clinical benefit from rescue tirofiban, including those with a PC stroke or a minor stroke. Accordingly, we assumed that the dosage of tirofiban may account for the clinical benefits in different stratified populations. Based on previously reported medication regimes of tirofiban in AIS patients undergoing EVT, we adopted an intra-arterial administration of < 1 mg and an intravenous infusion of 0.1 μg/kg/min for 12–24 h in patients refractory to recanalization (10). The present study demonstrated that this low- dose rescue tirofiban was effective in cases of AC stroke and major stroke. Nevertheless, since tirofiban was administered within the dosage range in our study, it might have different treatment effects in AIS patients under certain circumstances and may confound the therapeutic effects at a particular dose. Thus, further study with dose-escalation methods is needed for verification. In addition, the present study demonstrated that the use of tirofiban had more favorable outcome in anterior circulation strokes than in posterior circulation strokes. The possible postulated mechanisms attributed to this result may be due to the pathologic mechanisms of stroke and the fact that treatment modalities were significantly different in anterior and posterior circulation, which affect their clinical outcome (27). Posterior circulation stroke patients often presented severe preoperative symptoms and required longer emergency procedures, leading to poor neurological function recovery (27). In addition, the goal for rescue tirofiban is mainly to maintain blood flow and prevent acute occlusion. However, this issue remains uncertain and needs further large prospective trials or randomized controlled trials for verification.

This study had several limitations. First, an uneven proportion between the tirofiban and non-tirofiban groups may cause a bias. Second, the EVT and several other rescue therapies were undertaken at individual discretion, which might affect the treatment results. However, the indications triggering the use of rescue tirofiban were in accordance with standard clinical practice. Third, as the patients enrolled in this study were from China, the results cannot be generalized to the global population. Nonetheless, a strength of the current study was the relatively large sample size compared to previous studies. However, further randomized controlled trials are needed for verification.

CONCLUSIONS

Low-dose rescue tirofiban is safe in AIS patients with LAA, may provide clinical benefit to those with AC stroke or major stroke, and had a tendency to reduce the risk of mortality. However, large cohort or randomized controlled trials with dose-escalation are urgently needed for further verification.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committee of Beijing Tiantan Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZM, YlW, and YjW conceived and led the project. DM, FG and NM supervised and performed quality control for the study. AW performed statistical analysis, XH and R acquired the data and co-wrote the manuscript with input from all co-authors. All authors contributed to the article and approved the submitted version.

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Pre-treatment of Single and Double Antiplatelet and Anticoagulant With Intravenous Thrombolysis for Older Adults With Acute Ischemic Stroke: The TTT-AIS Experience

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Background: This study aimed to investigate the safety and efficacy of single antiplatelet, anticoagulant and Dual Antiplatelet pre-treatment (DAPP) in older, moderate to high severity acute ischemic stroke patients treated with intravenous thrombolysis (IVT).

Methods: A prospective cohort study was conducted to monitor the development of symptomatic intracranial hemorrhage (SICH) and functional outcomes at 90 days. Two different dosages of alteplase were used for IVT. Logistic regression models were used for analysis of the safety and efficacy outcomes.

Results: A total of 1,156 patients were enrolled and categorized into six groups based on their pre-treatment medications: (1) aspirin (n = 213), (2) clopidogrel (*n* = 37), (3) DAPP of aspirin + clopidogrel (*n*= 27), (4) warfarin (*n* = 44), (5) any of the above pre-medications (*n* = 331), and (6) none of these medications as controls (*n* = 825). The DAPP group showed significantly increased SICH by the NINDS (adjusted OR: 4.90, 95% CI 1.28–18.69) and the ECASS II (adjusted OR: 5.09, 95% CI: 1.01–25.68) standards. The aspirin group was found to significantly improve the favorable functional outcome of the modified Rankin Scale (mRS) of 0–1 (adjusted OR: 1.91, 95% CI, 1.31.2.78), but no significance for mRS of 0–2 (adjusted OR: 1.39, 95% CI, 0.97–1.99). The DAPP group also significantly increased mortality (adjusted OR: 4.75, 95% CI: 1.77–12.72). A significant interaction between different dosages for IVT and the functional status was noted. Compared to standard dose, the DAPP group showed higher proportions of disability and mortality with low dose of IVT.

Conclusion: For older adults with higher baseline severity of acute ischemic stroke, DAPP may increase the risk of SICH and mortality post IVT. However, DAPP is still not an indication to withdraw IVT and to prescribe low-dose IVT for older adults.

Keywords: aspirin, clopidogrel, stroke, intracranial hemorrhage, intravenous thrombolysis

INTRODUCTION

A *post hoc* analysis from the randomized controlled trial (RCT) of Enhanced Control of Hypertension and Thrombolysis Stroke (ENCHANTED) Study (1) indicated a significant interaction between the different doses of intravenous thrombolysis (IVT) and pre-treatment of antiplatelet (2). Compared to the standard dose of IVT with alteplase, the low-dose group showed increased favorable functional outcome after the pre-treatment with antiplatelets (2). They found that, the pre-treatment of antiplatelets with IVT revealed borderline significance for increased Symptomatic Intracranial Hemorrhage (SICH) (2) according to terms of the Safe Implementation of Thrombolysis in Stroke- Monitoring Study (SITS-MOST) criteria (3).

Previous studies on patients with acute ischemic stroke who were treated with IVT found a 2-fold risk of increased SICH with a single antiplatelet pre-treatment (4–6), and 4- to 9-fold increased risk with dual antiplatelet pre-treatment (DAPP) (4–6). This extremely high risk of increased SICH with DAPP was likely caused by selection bias. Two recent studies by Tsivgoulis et al. (7, 8) employed propensity score matching (PSM) to control the imbalance of the confounders between both the groups, with and without DAPP. Their results suggest that DAPP caused no significant increase in SICH by most standards except of the SITS-MOST criteria, and no significant improvement in the Favorable Functional Outcome (FFO) (7, 8). A recent pooled analysis study showed similar findings at first (9); however, a recent letter to this study revealed that the pooled results were biased by duplicate data and disproved the major findings (10). They indicated that DAPP significantly increased SICH by deleting duplicate data (10). Thereafter, some issues remain to be answered. First, studies employing the PSM method focused on mild ischemic stroke severity with a National Institute of Health Stroke Scale (NIHSS) score of $<$ 10 (7, 8). Second, there was a high heterogeneity in the definition of DAPP (including both aspirin + dipyridamole and aspirin + clopidogrel) and SICH standards in the pooled analysis (9). Third, we considered that older patients were more susceptible to bleeding with DAPP.

The aim of this study was to investigate whether pre-treatment with single antiplatelet, warfarin, and DAPP for acute ischemic stroke patients who were treated with IVT with the following characteristics: (1) older age, (2) moderate to high severity with high NIHSS score at baseline, and (3) the low-dose alteplase, imposed changed risk of SICH and the global functional outcomes.

METHODS

Study Design and Patients

The Taiwan Thrombolytic Therapy for Acute Ischemic Stroke (TTT-AIS) study was a multicenter, prospective cohort design, which was conducted between December 1, 2004 and December 31, 2016, throughout all regions in Taiwan. A detailed description of the data collection of TTT-AIS has been published previously (11–13). The TTT-AIS data sets include demographic characteristics, previous medical history, such as hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation, and alcoholism; time duration between stroke onset and IV thrombolysis; NIHSS at baseline; blood pressure at initial presentation; alteplase dose for IVT; levels of glucose, prothrombin time or international normalized ratio (INR), and activated partial thromboplastin time (aPTT) before IVT. All patients underwent brain computed tomography (CT) scans prior to IVT, and another brain CT scan was conducted within 24–36 h post IVT. The indications and contraindications for IVT were referred to the SITS-MOST (13) study except an upper age limit of 80 years. Patients treated with oral anticoagulant (including warfarin) with INR $>$1.7 was excluded for IVT.

Patients were eligible for enrollment if they met the following inclusion criteria: (1) age $\geq$ 60 years, (2) clinical diagnosis of acute ischemic stroke with treatment of intravenous thrombolysis within 3 h of stroke onset, and (3) information on the antiplatelets or anticoagulants used before the stroke onset between December 1, 2004 and December 31, 2016. Based on this data, we categorized these patients into the 6 premedication groups: (1) aspirin use (ASA), (2) clopidogrel or ticlopidine use (P2Y12), (3) dual antiplatelets of aspirin and clopidogrel use (DAPP), (4) warfarin use (WAR), (5) any antiplatelet or anticoagulant use (Any AP/AC), and (6) no use of antiplatelets or anticoagulants (no AP/AC). This study was approved by the Institutional Review Board of Kaohsiung Medical University (reference number: KMUH-IRB-20140305). Informed consent was obtained from the patients prior to their inclusion in the study.

Outcome Measures

For the safety outcome, two standards for SICH were used: (1) the National Institute of Neurological Disorders and Stroke (NINDS) criteria (14); intracranial hemorrhage with an increase of NIHSS $\geq$ 1 point or death within 36 h, (2) the European-Australasian Acute Stroke Study II (ECASS II); (14) intracranial hemorrhage with deterioration of NIHSS $\geq$ 4 point or death compared with baseline NIHSS within 36 h. Functional outcomes were assessed according to the modified Rankin Scale (mRS) (15). For the efficacy outcome, two definitions of better outcomes were employed: the favorable functional outcome (FFO) was defined as mRS of 0–1 at 90 days, and functional independence (FI) was taken as mRS of 0–2 at 90 days. Mortality (mRS of 6) at 90 days was also assessed.

Statistical Analysis

Continuous variables were compared using Student's *t*-test, while discrete variables were compared using the Chi-square or Fisher exact test. First, the associations among no AP/AC, prior AP/AC, and DAPP use on global functional outcome were analyzed using ordinal logistic regression analysis. Second, logistic regression was employed to estimate the OR (odds ratio) for outcome measures of SICH within 36 h of stroke onset and FFO, FI, and mortality at 90 days. The AP or AC naïve (No AP/AC) group was used as the control group. In addition, multivariate regression models were applied to adjust for the characteristic difference between the premedication group and no AP/AC group. Statistical significance was defined as (two-tailed) *P*-value < 0.05. All analysis were performed with the SAS 9.4 software (SAS Institute, Cary, NC, USA).

RESULTS

Characteristics of Enrolled Patients

A total of 1,156 patients aged $\geq$ 60 years were enrolled in this study (**Table 1**). Of these, 825 patients were categorized as having no AP/AC, and 331 were classified as having AP/AC. Of the any AP/ AC group, 213 patients were pre-treated with ASA, 37 with P2Y12, 27 with DAPP of ASA and clopidogrel, 44 with AC of warfarin, and 10 with other antiplatelets. Of the 10 patients, four patients were treated with dipyridamole and six stroke patients were with cilostazol, respectively (**Supplementary Table 1**). Of each group, the average age was in the range of 74–77 years. Around 40% of the patients were female. Overall, age and sex distribution in the six groups were in fact homogenous without significant differences. Regarding medical comorbidities, the ASA group had higher proportions of hypertension and diabetes, and the WAR group had higher proportions of atrial fibrillation. Both the P2Y12 and DAPP groups showed no significant difference in medical comorbidity compared to the no AP/AC group. Laboratory tests of glucose, INR, aPTT, and blood pressure showed no statistical significance. Moreover, patients in all six groups had moderate to high severity of acute ischemic stroke at baseline (mean NIHSS between 13 and 16), and 70% of patients were treated with low-dose alteplase for IVT. The onset-to-needle time was approximately 120 min for each group.

Distribution of Global Functional Outcomes by Dosage of Alteplase

Global function outcomes for the three groups of no AP/AC, any AP/AC, and DAPP are shown in **Figure 1**. Compared to standard-dose alteplase, low-dose alteplase presented no significant increase in the ordinal mRS for no AP/AC (OR: 1.27, 95% CI, 0.96–1.67), for any AP/AC (OR: 1.53, 95% CI, 0.93–2.52), and for the DAPP group (OR: 2.12, 95% CI, 0.39–11.67), respectively. However, a significant interaction was found between the dosage of alteplase and the use of DAPP ($P = 0.0113$). In the DAPP group, low-dose alteplase had a higher proportion of unfavorable functional outcomes and death.

Outcome Measures of Safety

The outcome measures for each pre-treatment of the AP/AC groups are shown in **Table 2**. Except for the DAPP group, the cumulative incidence of SICH was $\sim$2–5% by the NINDS and 1–3% by the ECASS II standards for each group, respectively. The DAPP group showed an extremely high cumulative incidence of SICH (11.1 and 7.4% according to the NINDS and ECASS II criteria, respectively). Pre-treatment with single antiplatelet agents of ASA or P2Y12 or anticoagulant of warfarin consistently showed no significant increase in SICH in both the simple and multivariate logistic regression models. In contrast, the DAPP group exhibited a significantly higher risk of SICH by the NINDS (OR: 5.61, 95% CI, 1.55–20.32, adjusted OR: 4.90, 95% CI, 1.28–18.69 in the adjusted model) and the ECASS II standards (OR: 5.61, 95% CI, 1.55–20.32 and adjusted OR: 4.90, 95% CI, 1.28–18.69), respectively. Of the 10 patients with other antiplatelets, no patients developed SICH (**supplementary Table 2**).

Outcome Measures of Efficacy

For outcomes of FFO of mRS of 0–1, the ASA group showed a greater number of patients with better functional outcome (38.3%) and the DAPP group showed a lower number of patients (18.2%). Among pre-treatment groups, the ASA group was the only one that showed significant improvements of FI (OR: 1.57, 95% CI, 1.12–2.21, and adjusted OR: 1.91, 95% CI, 1.31–2.78), respectively. Despite the DAPP group with a lower proportion of FFO, no significant difference was observed in the outcomes of FFO in comparison to the no AP/AC group. As for the FI of mRS of 0–2, the trend was similar to that of FFO, but no significant difference was found among pre-treatment groups. For outcome of mortality, each group had <10% mortality, except for the P2Y12 (15.6%) and DAPP groups (36.4%). Of them, the DAPP group showed a significant 4- to 5-fold risk of mortality (OR: 4.84, 95% CI, 1.96–11.94; adjusted OR: 4.75, 95% CI, 1.77–12.72).

DISCUSSION

In this study, we found a significant interaction between the dosage of alteplase and DAPP for the global functional outcome. In the DAPP group, low-dose alteplase had a higher proportion of unfavorable outcomes despite no significantly increased ordinal mRS. For the older adults, pre-treatment with ASA resulted in significant improvement of FFO but not FI. The DAPP group had lower proportions of better outcomes, but no significant difference in terms of FFO and FI compared to no AP/AC. However, DAPP was found to have a significantly increased 5-fold the risk of SICH with both NINDS and ECASS II standards. Mortality was found to be significantly increased by more than 4-fold in the DAPP group.

Our analysis was in line with most of the observational studies, which showed that prior dual antiplatelets increased

TABLE 1 | Characteristics of patients receiving antiplatelets and anticoagulants before intravenous thrombolysis (Total $N = 1,156$).

	ASA ($n = 213$)	P2Y12 ($n = 37$)	DAPP ($n = 27$)	WAR ($n = 44$)	Any AP/AC ($n = 331$)	No AP/AC ($n = 825$)
Age (years)	74.5 ± 7.9	76.6 ± 10.2	77.5 ± 7.5	74.3 ± 7.8	74.9 ± 8.3	74.9 ± 8.6
Age groups (years)						
60–69 years	30.5% (65/213)	32.4% (12/37)	18.5% (5/27)	34.1% (15/44)	30.8% (102/331)	30.2% (249/825)
70–79 years	41.3% (88/213)	27.0% (10/37)	44.4% (12/27)	36.4% (16/44)	39.0% (129/331)	39.6% (327/825)
80–89 years	24.4% (52/213)	29.7% (11/37)	33.3% (9/27)	27.3% (12/44)	25.7% (85/331)	24.9% (205/825)
≥90 years	3.8% (8/213)	10.8% (4/37)	3.7% (1/27)	2.3% (1/44)	4.5% (15/331)	5.3% (44/825)
Female sex (%)	39.0% (83/213)	32.4% (12/37)	48.2% (13/27)	43.2% (19/44)	39.9% (132/331)	39.5% (326/825)
Comorbidity (%)						
Hypertension	83.1% (177/213)**	83.8% (31/37)	59.3% (16/27)	65.9% (29/44)	78.9% (261/331)	74.4% (614/825)
Diabetes	44.6% (95/213)***	40.5% (15/37)	48.2% (13/27)	38.6% (17/44)	43.8% (145/331)***	30.6% (252/825)
Hyperlipidemia	27.2% (58/213)*	29.7% (11/37)	29.6% (8/27)	25.0% (11/44)	27.2% (90/331)**	35.9% (296/825)
Atrial fibrillation	46.6% (90/193)	45.7% (16/35)	53.9% (14/26)	90.2% (37/41)***	52.5% (159/303)***	40.0% (303/757)
Alcoholism	5.6% (12/213)	8.1% (3/37)	11.1% (3/27)	4.6% (2/44)	6.3% (21/331)	4.9% (40/825)
Glucose (mg/dl)	151.8 ± 53.9	156.0 ± 49.0	183.7 ± 95.5	141.9 ± 52.1	153.2 ± 57.7	148.4 ± 69.8
Prothrombin time (INR)	1.02 ± 0.11	1.00 ± 0.07	0.99 ± 0.11	1.13 ± 0.21**	1.03 ± 0.13	1.02 ± 0.10
aPTT	28.2 ± 4.8	28.1 ± 6.2	27.6 ± 4.0	29.8 ± 4.2	28.3 ± 4.8	29.7 ± 13.9
Systolic BP (mmHg)	158.2 ± 28.2**	159.1 ± 27.4	162.9 ± 29.5	152.4 ± 31.0	157.7 ± 28.5***	164.9 ± 29.6
Diastolic BP (mmHg)	88.9 ± 19.1*	87.9 ± 18.5	89.4 ± 18.3	88.9 ± 21.3	88.8 ± 19.0*	92.0 ± 19.2
Baseline NIHSS	13.1 ± 6.6	14.5 ± 7.0	15.5 ± 5.1	15.9 ± 7.7	13.8 ± 6.8	13.9 ± 7.2
Alteplase dose (mg/kg)	0.77 ± 0.15**	0.74 ± 0.17	0.80 ± 0.15	0.78 ± 0.15	0.77 ± 0.15***	0.81 ± 0.15
Standard dose	27.2% (58/213)	29.7% (11/37)	25.9% (7/27)	36.4% (16/44)	28.1% (93/331)	32.7% (270/825)
Low dose (<0.9 mg/kg)	72.8% (155/213)	70.3% (26/37)	74.1% (20/27)	63.6% (28/44)	71.9% (238/331)	67.3% (555/825)
Onset to needle time (min)	121.0 ± 59.8	130.5 ± 50.0	123.1 ± 55.8	105.0 ± 54.9	119.8 ± 58.3	120.8 ± 56.6

AC, anticoagulant; AP, antiplatelet; ASA, aspirin; aPTT, activated partial thromboplastin time; BP, blood pressure; DAPP, dual antiplatelet pre-treatment of aspirin and clopidogrel; INR, international normalized ratio; NIHSS, the National Institute of Health Stroke Scale; P2Y12, purinergic receptor P2Y12 inhibitor of clopidogrel and ticlopidine; WAR, warfarin.
P-values indicate comparison of each AP/AC vs. No AP/AC (Student's t-test for continuous variable and Pearson Chi-squared test for categorical variables).
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

the 4- to 9-fold risk of SICH (4–6). In contrast, our results showed some disagreement from two recent studies employing propensity score matching (PSM) adjustment (7, 8), which found that DAPP did not significantly increase SICH after adjustment for confounders. In fact, these studies employing PSM adjustment enrolled patients with mild severity of acute ischemic stroke (average NIHSS score of <10) (7, 8), while we included patients with higher severity (mean NIHSS score of 13–16). In addition, the control group showed different characteristics between these studies and ours (8). Their reference group included patients pre-treated with a single antiplatelet agent and no antiplatelets, whereas our reference group was treated without any AP/AC. Moreover, our patients were older. These characteristic differences between our enrolled patients and theirs, should cause the diverged results. On the other hand, the WAR group in our study showed no significant difference in comparison to no AP/AC. Since patients treated with warfarin with INR > 1.7 were excluded for IVT in our protocol, patients in the WAR group actually were below of their therapeutic range. This should explain the lower SICH rates in the WAR group.

Besides, this study revealed the real-world dosing patterns of IVT for eligible patients with acute ischemic stroke in Taiwan and other east Asian countries. In 2006, the Japan Alteplase Clinical Trial (J-ACT) showed equivalent efficacy and higher safety results of IV thrombolysis using alteplase at a dose of 0.6 mg/kg (16). Thereafter, the Japanese drug safety authority approved the dose. In 2010, an observational study in Taiwan (TTT-AIS) showed the standard dose of 0.9 mg/kg alteplase may not be optimal for aged population (11). In 2014, another study in Taiwan (TTT-AIS II) showed a lower dose of 0.6 mg/kg was associated with a better outcome for elderly (age group of 71–80 years) as well (12). In the background, most of the neurologists in Taiwan tended to adopt a lower dose of alteplase for IV thrombolysis during our enrollment. On the other hand, in 2018, an another analysis for Taiwanese octogenarian stroke patients with higher severity (high NIHSS score of ≥ 14 at initial presentation) showed a standard dose of 0.9 mg/kg with higher rates of the FFO in comparison to lower dose of 0.6 mg/kg (17). For mild stroke (NIHSS score of 4–8), both standard-dose and low-dose alteplase showed comparable rates of favorable functional outcomes, but low-dose alteplase for mild stroke

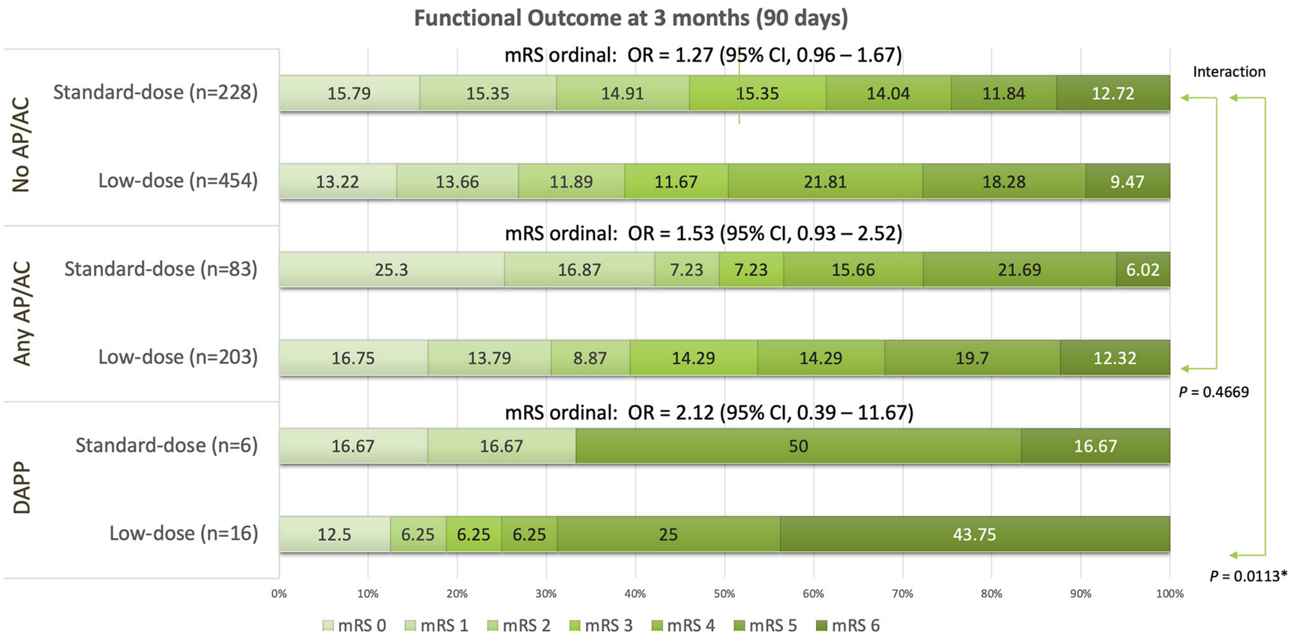

FIGURE 1 | Global functional outcomes at 90 days for groups of patients having pre-treatment without any antiplatelets or anticoagulants (no AP/AC), with any antiplatelets or anticoagulant (any AP/AC), and with dual antiplatelet (DAPP). *$P < 0.05$.

showed much reduced mortality on day 90 for octogenarians (17). In our study, the stroke patient characteristics (**Table 1**) showed higher baseline severity (mean NIHSS score of 13–15 for all pre-treatment groups). We considered a lower dose should be inadequate for the stroke patients with higher severity patients and the results showed higher death rates.

Our inclusion of stroke patients of >60 years for IVT should be representative of the majority stroke patients in Taiwan. The demographic data in Taiwan showed the incidence rates of first stroke for age below 60 years were extremely low (18, 19). Among the largest cohort of 8,562 stroke-free people in Taiwan followed for 4 years, the incidence rate of first stroke for age groups of 36–44 and 45–54 years were 2 per 100,000 and 12 per 100,000 person-years, respectively (18). The incidence rate of first stroke throughout all age groups was 104 per 100,000 person-years (18). In contrast, the incidence rate of first stroke for age groups of 55–64, 65–74, and over 75 years were, respectively, 41 per 100,000, 29 per 100,000 and 20 per 100,000 years (18). The proportion of first stroke incidence rate for age below 55 years in Taiwanese population was 13.5%.

The propensity score matching was not conducted in our study due to our multiple pre-treatment groups (five groups). Currently, methods of binary treatment (two groups) propensity scoring are well-developed and established completely (20–22). Methods of multinomial treatment (>2 groups) propensity scoring have been in the developing status and were increasing unstable with expanding the comparison groups since the current distance-based matching approach cannot be extended to more than three groups (21, 23–25). Since we had five pre-treatment groups of ASA, P2Y12, DAPP, WAR, and any AP/AC, using the method of binary treatment propensity score matching would produce five reference groups of No AP/AC. The demographic and characteristic composition among the new five reference groups for those ASA, P2Y12, DAPP, WAR, and any AP/AC were totally different. The efficacy and safety outcome among the five pre-treatment groups were not comparable. To reasonably comparing with the five pre-treatment groups, we preferred to use method of multivariate logistic regression since they shared the same reference group.

Our studies have robust strengths and offer distinctive information. First, the patients enrolled were homogenous for the baseline demographic and medical characteristics among the six groups, such as age, sex, laboratory tests, alteplase dose, and the onset to needle time. Second, patients who were enrolled in the study had moderate to high baseline severity of acute ischemic stroke. Third, we found significant interactions between different doses of alteplase and DAPP. Fourth, we focused on older patients. This should offer the evidence that DAPP should still be cautious for older adults with acute ischemic stroke treated with IVT. Lastly, TTT-AIS was a multicenter study across all regions in Taiwan, and the representative nationwide cohort was used for analysis (11–13). This study investigated the efficacy and safety of antithrombotic pre-treatment in real-life study model.

This study has some limitations. First, the prospective cohort study design in our study was still susceptible to residual confounding. Some unmeasured confounding effects were not controlled. In addition, the analysis in this study excluded patients aged <60 years, and these findings may not be applicable

TABLE 2 | Functional Outcome at 3 months (3 m).

Functional outcomes	ASA (n = 188)	P2Y12 (n = 32)	DAPP (n = 22)	WAR (n = 36)	Any AP/ AC (n = 286)	No AP/ AC (n = 682)
SYMPTOMATIC INTRACRANIAL HEMORRHAGE (SICH)						
SICH per NINDS						
n/ total *n* (%)	3.3% (7/213)	5.4% (2/37)	11.1% (3/27)	2.3% (1/44)	3.9% (13/331)	2.2% (25/1,139)
OR (95% CI)	1.52 (0.63–3.70)	2.56 (0.57–11.48)	5.61 (1.55–20.32)**	1.04 (0.14–7.99)	1.83 (0.89–3.79)	Ref. group
Adjusted OR†	0.99 (0.38–2.59)	1.95 (0.43–8.84)	4.90 (1.28–18.69)*	0.83 (0.19–6.94)	1.34 (0.62–2.90)	Ref. group
SICH per ECASS II						
n/total *n* (%)	2.8% (6/213)	2.7% (1/37)	7.4% (2/27)	2.3% (1/44)	3.0% (10/331)	1.3% (11/825)
OR (95% CI)	2.15 (0.78–5.87)	2.06 (0.26–16.36)	5.92 (1.25–28.13)*	1.72 (0.22–13.64)	2.31(0.97–5.48)	Ref. group
Adjusted OR†	1.38 (0.46–4.16)	1.68 (0.21–13.69)	5.09 (1.01–25.68)*	1.97 (0.21–18.75)	1.70(0.71–4.51)	Ref. group
FAVORABLE FUNCTIONAL OUTCOME (FFO)						
mRS of 0–1 at 90 days						
n/ total *n* (%)	38.3% (72/188)	28.1% (9/32)	18.2% (4/22)	30.6% (11/36)	33.9% (97/286)	28.3% (193/682)
OR (95% CI)	1.57 (1.12–2.21)**	0.99 (0.45–2.18)	0.56 (0.19–1.69)	1.12 (0.54–2.31)	1.30 (0.97–1.75)	Ref. group
Adjusted OR†	1.91 (1.31–2.78)***	1.20 (0.51–2.82)	0.66 (0.21–2.06)	1.03 (0.43–2.45)	1.51 (1.08–2.10)	Ref. group
FUNCTIONAL INDEPENDENCE (FI)						
mRS of 0–2 at 90 days						
n/total *n* (%)	45.2% (85/188)	34.4% (11/32)	22.7% (17/22)	50.0% (18/36)	42.3% (121/286)	41.2% (281/682)
OR (95% CI)	1.18 (0.85–1.63)	0.75 (0.36–1.58)	0.42 (0.15–1.15)	1.43 (0.73–2.79)	1.05 (0.79–1.38)	Ref. group
Adjusted OR†	1.39 (0.97–1.99)	0.94 (0.42–2.08)	0.54 (0.19–1.55)	1.59 (0.75–3.39)	1.19 (0.87–1.62)	Ref. group
Death at 90 days						
n/ total *n* (%)	7.5% (14/188)	15.6% (5/32)	36.4% (8/22)	8.3% (3/36)	10.5% (30/286)	10.6% (72/682)
OR (95% CI)	0.68 (0.38–1.24)	1.57 (0.59–4.20)	4.84 (1.96–11.94)***	0.77 (0.23–2.58)	0.99 (0.63–1.56)	Ref. group
Adjusted OR†	0.68 (0.36–1.29)	1.92 (0.70–5.32)	4.75 (1.77–12.72)**	0.83 (0.24–2.90)	0.99 (0.61–1.61)	Ref. group

AC, anticoagulant; AP, antiplatelet; ASA, aspirin; DAPP, dual antiplatelet pre-treatment of aspirin and clopidogrel; ECASS II, the European-Australasian Acute Stroke Study II; FFO, favorable functional outcome; FI, functional independence; mRS, modified Rankin Scale; P2Y12, purinergic receptor P2Y12 inhibitor of clopidogrel and ticlopidine; NINDS, National Institute of Neurological Disorders and Stroke Study; SICH, symptomatic intracranial hemorrhage; War, warfarin; mRS, modified Rankin Scale; OR, odds ratio.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.*
†Model adjusted for hypertension, diabetes, hyperlipidemia, atrial fibrillation, and alteplase dose.

throughout all ages. Second, there were a small number of patients in the DAPP group (2.3%, $n = 27$) and therefore the outcomes of SICH per NINDS (three out of 27 patients, adjusted OR: 4.90, 95% CI 1.28–18.69) and per ECASS II (two out of 27 patients, adjusted OR: 5.09, 95% CI: 1.01–25.68) in the DAPP group showed wide confidence interval. Although the number of DAPP patients in this study was small, this low proportion reflects real-world conditions. Previous observational studies indicated that the proportion of DAPP ranged from 1.3 to 7.3% for all ischemic stroke patients treated with IVT. Third, warfarin group was not representative to those patients treated with sufficient dose of warfarin. Only stroke patients insufficiently treated with warfarin (INR < 1.7) and IVT were included for this analysis. Lastly, some patients had incomplete follow-up at 90 days. In Taiwan, the prolonged hospital stay and readmission for stroke patients are serious problems. Since March 2014, the nationwide post-acute stroke care (PAC) program (26) was launched to improve the problems of shortage of acute beds, and overcrowded emergency departments. Stroke patients with stable neurological functional status for ≥72 h and no uncontrolled complications were transferred to regional hospitals. Some patients participating in the PAC program were unwilling to be contacted. While the follow-up rates for groups of ASA, P2Y12, DAPP, and WAR groups were 88.3% (188/213), 86.5% (32/37), 81.5% (22/27), and 81.8% (36/44), the follow-rate for no AP/AC group was 82.7% (682/825). Theoretically, no differential incomplete follow-up (27) were found between groups with and without AP/AC pre-treatment, and the validity in our analysis was still assured.

In clinical practice, physicians should be cautious for older patients receiving DAPP before IVT. Since a significant interaction for functional outcome was noted between the different dosage of alteplase and use of DAPP (**Figure 1**), standard-dose alteplase still could be considered for these stroke patients with high-risk and higher baseline severity. In our previous analysis for 249 old stroke patients over the age of 80 years, standard-dose alteplase was associated with an increased proportion of FI of mRS 0–2 (34.8 vs. 22.2%) and a little increased mortality (13.5 vs. 9.3%) at 90 days (17). Nevertheless, the previous subgroup analysis for a total of 128 octogenarian patients of high severity (NIHSS $\geq$ 14) showed an increased proportion of FI of mRS 0–2 (20.8 vs. 8.9%) and a near equivalent mortality (15.3 vs. 14.3%) (17). Although an early observational study of IVT in Japan showed similar functional outcomes and approved a low-dose IVT of 0.6 mg/kg (16), we did not recommend universal use of low-dose IVT for all older patients. Consequently, physicians should evaluate many factors of age, baseline stroke severity, comorbidities, and pre-treatment of antiplatelets and anticoagulants when prescribing the optimal dose of alteplase for acute ischemic stroke patients.

CONCLUSION

In conclusion, pre-treatment with ASA seems to improve functional outcomes in terms of FFO (mRS of 0–1), but not of FI (mRS 0–2). For older adults, DAPP increases the risk of SICH, especially for patients presenting themselves with moderate to high severity of acute ischemic stroke following IVT. In addition, DAPP increased the risk of mortality for older adults and showed no increase for the better outcomes in terms of FFO and FI. Nevertheless, DAPP still should not be the reason to hold IVT and to prescribe low-dose IVT in our analysis.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because distribution of dataset was restricted by Institutional Review Board of Kaohsiung Medical University. Requests to access the datasets should be directed to A-Ching Chao, achch@cc.kmu.edu.tw.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Kaohsiung Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

S-FL wrote the first draft of the manuscript. All authors contributed to the conception and design of the study, acquisition, analysis, and interpretation of data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.628077/full#supplementary-material

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MINI REVIEW
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Antiplatelet Therapy in the Secondary Prevention of Non-cardioembolic Ischemic Stroke and Transient Ischemic Attack: A Mini-Review

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The aim of this mini-review is to discuss the main antiplatelet agents that have been successfully used in the secondary prevention of non-cardioembolic ischemic stroke and transient ischemic attacks (TIA). The methodology is based on a literature review of available peer-reviewed English studies listed in PubMed. The findings reveal that aspirin remains a reliable antiplatelet agent in the secondary prevention of acute non-cardioembolic ischemic stroke and TIA. Nevertheless, currently, there are also other agents, i.e., ticagrelor, clopidogrel, and cilostazol, that can be applied. In addition, the results indicate that time is significant not only in severe stroke but also in non-severe stroke and TIA, which suggests that antiplatelet therapy should be applied within 24 h after the first symptoms because early treatment can lead to an improvement in neurological outcomes and reduce the chance of an early subsequent stroke.

Keywords: ischemic stroke, transient ischemic attack, aspirin, clopidogrel, cilostazol, ticagrelor, dipyridamole, antiplatelet therapy

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INTRODUCTION

Strokes are among the major causes of morbidity, mortality, and long-term disability (1). As stated by Béjot et al. (2), monthly mortality from strokes in Europe ranges from 13 to 35%. In addition, in European Union countries, the number of patients with strokes is expected to grow by 27% between 2017 and 2047. The reason for this is that there has been an increase in aging population groups and better survival rates after a stroke (3).

The most frequent type of stroke, comprising on average between 80 and 90% of all strokes, is ischemic stroke (4). This type of stroke results from the occlusion of the artery that supplies blood to the brain. The occlusion decreases blood flow and oxygen to the brain, contributing to harm or death of brain cells. If the circulation is not reestablished quickly, the brain damage can be permanent (5). The severity of ischemic stroke ranges from clinically mild (i.e., a minor stroke or transient ischemic attack [TIA]) to very severe (i.e., a major ischemic stroke), but the underlying causes are identical (6). The initial manifestations of ischemic stroke and TIA are often followed by recurrent vascular events, including recurrent strokes (7).

At present, antiplatelet therapy is crucial in the management of non-cardioembolic ischemic stroke and TIA, representing approximately 80% of all acute ischemic cerebrovascular events (8), and in their prevention (9), which is very important since repeated strokes occur in 10–20% of patients within 3 months after the first stroke (1).

Antiplatelet agents are usually preferred over anticoagulant agents due to their connection with lower rates of intracranial hemorrhage and moderately lower global death rates. Antiplatelet monotherapy is usually favored over dual antiplatelet therapy (DAPT) because DAPT is often associated with severe bleeding complications (10). The guidelines of the American Heart Association and American Stroke Association suggest antiplatelet monotherapy with aspirin within 24–48 h of an acute ischemic stroke. The recommended doses range between 160 and 300 mg per day (11). Despite aspirin being suggested as therapy for acute ischemic stroke, it does not succeed in preventing platelet aggregation in 5–55% of patients (12). Nevertheless, the administration of DAPT immediately following a minor ischemic stroke (NIHSS score of ≤3) or high-risk TIA (ABCD2 score of ≥4) may be beneficial and outweigh the risks in patients with acute minor ischemic stroke or high-risk TIA. In this respect, the guidelines suggest 21-day treatment with aspirin and clopidogrel to be started within 24 h of symptoms onset with minor stroke. However, this combination should not be administered immediately after intravenous thrombolysis (IVT) (11).

Furthermore, two independent multicenter, randomized, double-blind, placebo-controlled trials have established the efficacy of short-term DAPT to prevent recurrent ischemic stroke in patients with minor stroke or high-risk TIA. The CHANCE trial (Clopidogrel in High-Risk Patients with Acute Non-disabling Cerebrovascular Events) in the Chinese population demonstrated a 32% reduction in recurrent stroke at 90 days (ischemic or hemorrhagic) with no increase in major bleeding (13). In the POINT trial (Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke), conducted in North America, Europe, Australia, and New Zealand, up to 90 days after the index event, DAPT was associated with a 28% reduction in ischemic stroke (which was higher during the first 30 days of treatment) but was also associated with a higher number of major hemorrhages (14). However, in both trials (CHANCE and POINT), the loading dose of clopidogrel was given before the initiation of DAPT. In addition, researchers have also found that DAPT (aspirin with clopidogrel) may be ineffective in 5–30% of patients with percutaneous coronary interventions, mostly due to clopidogrel resistance. This number increases to 66% in patients with neuro-interventional events (15). Recently, Steffel et al. (16) discovered that rivaroxaban plus aspirin was associated with fewer adverse cardiovascular events but more major bleeding events than aspirin alone.

To date, clinical studies have provided evidence about four antiplatelet agents: aspirin, aspirin-dipyridamole, clopidogrel, and ticagrelor (9), which have also been widely described in clinical practice guidelines (11).

The purpose of this mini-review is therefore to provide an update on the main antiplatelet agents that have been successfully used in the secondary prevention of non-cardioembolic ischemic stroke and TIA, as well as to explore whether novel and effective antiplatelet agents and strategies have occurred in the secondary prevention of non-cardioembolic ischemic stroke and TIA.

METHODS AND MATERIALS

The authors of this mini-review conducted a literature search of peer-reviewed English language research articles listed in PubMed. The articles were searched using the following keywords: *antiplatelet therapy* AND *stroke*, *antiplatelet therapy* AND *ischemic stroke*, *antiplatelet agents* AND *ischemic stroke*, *antiplatelet therapy* AND *transient ischemic attack, antiplatelet agents* AND *transient ischemic attack.* The search was performed for studies published between January 1, 2015, and August 31, 2020, because several review studies on this topic summarized the literature prior to this period [cf. (17–19)]. The authors also conducted a backward search, i.e., they searched the references of the studies identified for relevant research studies that could have been missed during the PubMed searches. Only randomized controlled studies were included in the final analysis and evaluation.

RESULTS

Altogether, 11 randomized controlled trials (RCTs) were detected in PubMed and during the backward searches of the reference lists of the selected studies. Four studies originated in Japan, two in China, one in the UK, one in the USA, and one in Thailand, and two were multinational RCTs. The number of patients ranged from 21 to 13,199. The intervention period lasted from 1 week to 4 years. In all studies, aspirin was used either alone or with another antiplatelet drug. The most common were clopidogrel and cilostazol. The remaining antiplatelet drugs included in the studies were ticagrelor and dipyridamole. Cilostazol was exclusively used in studies of Asian origin, while clopidogrel was used across different continents. **Table 1** below provides the basic characteristics of these drugs apart from the use of dipyridamole, which is now considered to be obsolete.

Furthermore, the use of individual antiplatelet agents and their combinations in the detected studies are described below.

Monotherapy: A recent study states that dual therapy is more beneficial than monotherapy. This corresponds to the fact that in the literature in recent years, we found very few studies of monotherapy. Amarenco et al. (7) demonstrated the superiority of ticagrelor (6.7%; $p = 0.017$) over aspirin (9.6%) in the prevention of stroke, myocardial infarction, and death after aspiration (9.6%) in patients with acute ischemic stroke or a transient ischemic attack at 90 days (HR 0.68; 95% CI 0.53–0.88; $p = 0.003$). Haungsaithong et al. (22) evaluated changes in mean platelet volume (MPV) after the use of four antiplatelet drugs (aspirin, clopidogrel, aspirin plus dipyridamole and cilostazol) in patients with acute non-cardioembolic ischemic stroke to assess the effect of antiplatelet therapy and MPV on the stroke outcome. The authors concluded that after 4 weeks of therapy, MPV was reduced, as well as the NIHSS score, but only clopidogrel reduced this score with statistical significance ($p = 0.003$).

Dual therapy: aspirin plus clopidogrel—this combination is the most common in the literature. Jing et al. (23) divided patients into three research subgroups (those with multiple acute infarctions, single acute infarctions and no acute infarctions) and compared the effect of dual therapy and monotherapy in

TABLE 1 | Antithrombotic agents: platelet aggregation inhibitors (B01AC) (20, 21).

Group of drugs	Antithrombotic agents: platelet aggregation inhibitors excl. heparin			
Active agent	acetylsalicylic acid	clopidogrel	cilostazol	ticagrelor
ATC classification	B01AC06	B01AC04	B01AC23	B01AC24
Mechanism of action	Irreversible inhibition of COX-1 cyclooxygenase, thereby preventing the synthesis of thromboxane A2 in platelets and inhibiting their aggregation.	Irreversible selective inhibition of ADP binding to the platelet P2Y12 receptor and subsequent ADP-mediated activation of the GPIIb/IIIa glycoprotein complex, thereby inhibiting platelet aggregation.	Reversible inhibition of platelet aggregation.	Direct selective reversible antagonism of the P2Y12 receptor, which prevents platelet activation and aggregation
Active compound	Salicylic acid	SR26334	–	–
Half-time (h)	2–3 h (low doses)	8 h	10.5 h	1.5 h
Tmax (h)	From 10–20 min (acetylsalicylic acid) to 120 min (total salicylate)	30–60 min	–	2–4 h
Dose per day according to WHO (1)	1 tablet independent of strength (2)	75 mg p.o.	200 mg p.o.	180 mg p.o.
Most common side effects	Stomach pain, nausea, vomiting, diarrhea, GIT microhemorrhage, skin reactions.	Gastrointestinal bleeding, diarrhea, abdominal pain, dyspepsia, hematomas, epistaxis.	Headache, diarrhea, abnormal stools.	Bleeding and shortness of breath.

(1) The DDDs are based on prophylaxis for thrombosis. (2) The DDD of acetylsalicylic acid is given as 1 tablet independent of tablet strength. This is due to the great variations between different countries in the dosages/strengths recommended for the prophylaxis of thrombosis.

these groups in terms of recurrent stroke. In the group with multiple acute infarctions, recurrent stroke occurred in the following percentage of patients: 10.1 (dual therapy) and 18.1% (aspirin alone), i.e., HR, 0.5; 95% CI, 0.3–0.96 ($p = 0.04$). In the group with single acute infarctions, no difference between dual therapy and aspirin alone was observed, i.e., 8.9 vs. 8.5% (HR, 1.1; 95% CI, 0.6–2.0; $p = 0.71$). For the no acute infarctions group, the results were as follows: 2.6 (dual therapy) vs. 1.4% (aspirin alone), i.e., HR, 1.7; 95% CI, 0.3–11.1 ($p = 0.56$). The authors concluded that dual clopidogrel and aspirin therapy appears to have the most significant clinical benefit for patients with multiple acute infarctions. Johnston et al. (14), based on a clinical study, reported that dual therapy vs. aspirin alone was associated with a lower risk of a severe ischemic event of 5.0 vs. 6.5%, i.e., HR 0.75; 95% CI, 0.59–0.95 ($p = 0.02$). He et al. (24) examined a group of patients with minor stroke or transient ischemic attack (TIA) to compare dual therapy vs. aspirin monotherapy for neurological deterioration, recurrent stroke, or stroke development in patients with a TIA within 14 days of admission. During the 2-week period, worsening of stroke occurred in nine patients in the dual therapy group and 19 patients in the monotherapy group. Stroke occurred after the TIA in one patient in the dual therapy group and in three patients in the monotherapy group. The authors concluded that early dual therapy can reduce neurological deterioration in patients with acute ischemic stroke as compared to monotherapy.

Dual therapy: aspirin plus cilostazol—two current studies can be found in the literature for this combination. Ohnuki et al. (12) found no significant differences in platelet aggregation, platelet activation, or endothelial biomarker levels in patients receiving dual therapy compared to the aspirin group. Aoki et al. (25) reported no significant differences between dual therapy and aspirin alone. The aim of this study was to determine the effectiveness of both therapies in patients with non-cardioembolic stroke within 48 h of the onset of symptoms. The treatment was evaluated as safe but failed to reduce the rate of short-term neurological worsening.

Dual therapy: aspirin plus ticagrelor—Amarenco et al. (26) evaluated the combination of ticagrelor and aspirin (as opposed to aspirin alone) in stroke prevention. The main endpoint was the time to stroke (progression of the event or a new stroke) or death within 30 days. Disabling stroke was defined by a modified Rankin Scale score (mRS) >1. A score of mRS > 1 occurred in the following percentage of patients: 4.0 (dual therapy) vs. 4.7% (aspirin alone), i.e., HR, 0.83; 95% CI, 0.69–0.99 ($p = 0.04$). Based on this study, it appears that ticagrelor added to aspirin was superior to aspirin alone in preventing disabling stroke or death at 30 days and reduced the total burden of disability owing to ischemic stroke recurrence.

The key findings reveal that DAPT appears to be safe and efficient in decreasing the risk of multiple ischemic strokes, particularly in the long term and with larger sample sizes (27), as well as being more effective than monotherapy in the early stages of acute ischemic stroke (24). This has also been confirmed by the most recent study by Amarenco et al. (26), in which patients with TIA and minor ischemic stroke received ticagrelor in combination with aspirin to prevent disabling stroke or death at 30 days. The results showed that DAPT was more efficient than aspirin alone in decreasing the total burden of disability due to ischemic stroke recurrence. However, the results differed in the risk of hemorrhage. While Jing et al. (23) and Toyoda et al. (27) confirmed a reduced risk of severe bleeding after 3 months, Johnston et al. (14) found the opposite result. Furthermore, the results indicated that the triple combination had no gains with respect to the occurrence and severity of recurrent stroke but actually had a higher rate of hemorrhage (28).

DISCUSSION

Our review of the literature revealed that aspirin is a reliable antiplatelet agent in the secondary prevention of acute non-cardioembolic ischemic stroke and TIA and that it is the only drug that has received a class 1A recommendation (11). However, Amarenco et al. (7), within the SOCRATES project, indicated that if ticagrelor was administered within 24 h of symptom onset, it would be more effective than aspirin in preventing stroke, myocardial infarction, or death at 90 days in patients with acute ischemic stroke or a transient ischemic attack when associated with potentially symptomatic ipsilateral atherosclerotic stenosis. Their findings also showed that there were no differences in the rate of life-threatening bleeding or major or minor bleeding episodes in patients with ipsilateral stenosis in the ticagrelor group compared with the aspirin group.

Haungsaithong et al. (22) reported that clopidogrel considerably decreased the NIHSS score ($p = 0.003$), and it resulted in the greatest reduction in MPV compared with the others. However, their study was quite small and followed the patients for only 4 weeks. Nevertheless, their findings were also confirmed by Paciaroni et al. (29) in their recent meta-analysis, in which they revealed smaller risks of severe undesirable cardiovascular or cerebrovascular episodes, recurrent stroke, and bleeding episodes for clopidogrel monotherapy compared to aspirin. These results confirmed the clinical benefits of antiplatelet monotherapy with clopidogrel over aspirin for secondary prevention in patients with a recent ischemic stroke.

In secondary prevention, dual antiplatelet therapy appears to be the best solution. A combination of aspirin and clopidogrel seems to be beneficial not only shortly (24 h to 1 week) after an acute non-cardioembolic stroke, most often a minor ischemic stroke, or a high-risk TIA (24) but also throughout the first 90 days after the event (14, 23, 27). These findings have also been confirmed by other studies (30, 31). Rahman et al. (30) stated that DAPT with aspirin and clopidogrel significantly reduced the risk of recurrent IS in the short-term (RR, 0.53; 95% CI, 0.37–0.78) and intermediate-term (RR, 0.72; 95% CI, 0.58–0.90). In addition, their findings also proved that intermediate-term (RR, 2.58; 95% CI, 1.19–5.60) and long-term (RR, 1.87; 95% CI, 1.36–2.56) aspirin and clopidogrel regimens significantly increased the risk of major bleeding compared to short-term aspirin and clopidogrel (RR, 1.82; 95% CI, 0.91–3.62), as revealed by Johnston et al. (14). The same findings were reported by Greving et al. (32). In their meta-analysis, they stated that a combination of clopidogrel and aspirin is beneficial for long-term secondary prevention after a non-cardioembolic stroke or transient ischemic attack, regardless of the patient characteristics. However, this combination is connected with a considerably higher risk of major bleeding than other DAPTs. Hao et al. (33) further found that DAPT with aspirin and clopidogrel administered within 24 h after high-risk TIA or minor ischemic stroke decreases the incidence of subsequent stroke by ∼20 in 1,000 people, with a possible increase in moderate to severe bleeding of 2 per 1,000 population.

Furthermore, the evaluation of the detected trial (28) revealed that triplet antiplatelet therapy did not have any superiority over DAPT regarding the recurrence of strokes. In contrast, the rate of hemorrhage was much higher in these patients than in those who received fewer medications.

We also found that monotherapies with aspirin or ticagrelor and DAPT with aspirin and clopidogrel are mainly used in western Europe and the USA, while cilostazol, either alone or combined with aspirin, is predominantly used in Asia [cf. (34)]. However, the experts assume that there is no reason to test cilostazol in Europe and the USA since it has a good safety profile and seems safer than aspirin and is probably safer than other antithrombotic drugs in terms of reducing bleeding complications, especially hemorrhagic strokes.

The limitations of the included studies include their different sample sizes, various lengths of intervention periods, sometimes slightly different dosages of antiplatelet drugs, and an absence of sufficiently long follow-up periods in some studies.

Overall, the findings reveal that aspirin is a reliable antiplatelet agent in the secondary prevention of acute non-cardioembolic ischemic stroke and TIA. Nevertheless, currently, there are also other agents, i.e., ticagrelor, clopidogrel, and cilostazol, that can be applied. In addition, the results indicate that time is significant not only in severe stroke but also in non-severe stroke and TIA, which suggests that antiplatelet therapy should be administered within 24 h after the first symptoms (after 24 h in patients treated with IVT) because early treatment can lead to an improvement in neurological outcomes and a decrease in the risk of early subsequent stroke.

Future research should focus on identifying more effective drugs that could be developed for use in monotherapy because dual therapy only increases the rate of adverse events related to polypharmacy. In addition, these novel molecules could increase the risk of excessive bleeding. In fact, currently, a few studies are being conducted on this topic (**Table 1**).

AUTHOR CONTRIBUTIONS

MV, BK, MN, and RH equally contributed to the whole concept of the article, its methodology, data processing, and drafting and revision. All authors agreed with this final version of the manuscript.

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Clopidogrel Plus Aspirin in Patients With Different Types of Single Small Subcortical Infarction

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Background: We aim to investigate the effects and safety of clopidogrel plus aspirin in patients with different types of single small subcortical infarction (SSSI) in the Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Events (CHANCE) trial.

Methods: SSSI was defined as single DWI lesion of ≤2.0 cm. Patients with SSSI were divided into SSSI + PAD (parent artery disease) and SSSI – PAD, according to the stenosis of the parent artery. The efficacy outcome was stroke recurrence during 90-day follow-up. Cox proportional hazards models or logistic regression models were used to assess the interaction of the treatment effects of clopidogrel plus aspirin vs. aspirin alone among patients with and without PAD.

Results: Among 338 patients with SSSI included in the subanalysis, 105 were with PAD and 233 without. The efficacy of clopidogrel plus aspirin compared with aspirin alone on any stroke was consistent between patients with [adjusted hazard ratio (HR) 0.84; 95% confidence interval (CI), 0.25–2.75] and without PAD (adjusted HR 1.03; 95% CI, 0.40–2.68, interaction $P = 0.83$). In patients with SSSI + PAD, the rate of stroke recurrence in those treated with dual antiplatelet therapy and mono antiplatelet therapy was not significantly different (10.9 vs. 13.6%, $P = 0.77$). The number of bleeding events was similar between the clopidogrel-aspirin group and aspirin group regardless of SSSI + PAD or SSSI – PAD.

Conclusions: There was no significant difference in the efficacy of clopidogrel plus aspirin compared with aspirin alone between patients with SSSI + PAD and SSSI – PAD in the CHANCE trial. Studies in other populations and with adequate power are needed to further verify such findings.

Keywords: SSSI, PAD, lesion location, dual antiplatelet, prognosis

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INTRODUCTION

Single small subcortical infarction (SSSI), commonly known as lacunar stroke, is an important ischemic stroke (IS) subtype (1–5), and accounts for 25% of all IS in westerners (6) and 16.8–42.0% in Chinese (7–9). A recent study showed that SSSI in perforator territory had a heterogeneous pathogenesis regarding the presence of parental arterial disease (PAD) and SSSI associated with

PAD (SSSI + PAD) had higher prevalence of atherosclerosis than those without PAD (SSSI – PAD) (10). SSSI – PAD were probably related to fibrinoid necrosis or "lipohyalinosis" of small perforating arteries (11).

At present, little is known about the optimal antiplatelet strategy for early stroke prevention in patients with lacunar strokes. The Secondary Prevention of Small Subcortical Strokes (SPS3) trial has found that the addition of clopidogrel to aspirin did not significantly reduce the risk of recurrent stroke but significantly increased the risk of bleeding and death among patients with recent lacunar strokes (12). The second Cilostazol Stroke Prevention Study (CSPS 2) showed Cilostazol seemed not to be inferior to aspirin for the prevention of stroke after lacunar stroke (13).

Several studies have indicated that dual antiplatelet therapy with clopidogrel and aspirin was an effective treatment in patients with symptomatic carotid or intracranial arterial stenosis compared to aspirin alone (hereinafter, "mono antiplatelet therapy") (14–16). Considering the heterogeneous pathogenesis of SSSI, whether IS patients with different types of SSSI based on the presence or absence of PAD might benefit from dual antiplatelet therapy is still uncertain.

In the Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Events (CHANCE) trial, clopidogrel plus aspirin reduced the risk of recurrent stroke in Chinese patients with acute minor stroke or high-risk transient ischemic attack (TIA). Therefore, we aim to investigate whether different types of SSSI (SSSI + PAD or SSSI – PAD) can benefit from dual antiplatelet therapy in this subgroup analysis of CHANCE trial.

METHODS AND MATERIALS

Study Design

The design and main results for CHANCE trial have been described previously (9, 17). In brief, a total of 5,170 patients from 114 clinic centers within 24 h after the onset of minor ischemic stroke or high-risk TIA to combination therapy with clopidogrel and aspirin (clopidogrel at an initial dose of 300 mg, followed by 75 mg per day for 90 days, plus aspirin at a dose of 75 mg per day for the first 21 days) or to placebo plus aspirin (75 mg per day for 90 days). CHANCE was registered with ClinicalTrials.gov, identifier NCT00979589. The imaging subgroup study was approved by the ethics committees of all participating centers.

Efficacy and Safety Outcomes

The primary outcome of the CHANCE trial was stroke (ischemic or hemorrhagic) during the 90-day follow-up in an intention-to-treat analysis. Secondary efficacy outcomes included a new clinical vascular event at 90 days (IS, hemorrhagic stroke, myocardial infarction, or vascular death)—analyzed as a composite outcome and individual outcomes as well, and disabling/fatal stroke (modified Rankin Scale score of 2 to 6 at 90 days). The primary safety outcome was a moderate-to-severe bleeding event at 90 days, as per the Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) definition (18). Severe bleeding event was defined as a fatal or intracranial hemorrhage or other hemorrhage causing hemodynamic compromise requiring treatment. Moderate bleeding event was defined as bleeding requiring blood transfusion. Other safety outcomes included mild bleeding by the GUSTO definition and any bleeding event.

Subjects

Of 5,170 patients enrolled in CHANCE, 1,089 consecutive patients participated in the imaging subgroup study. All MRI/MRAs of the brain were performed within 7 days of symptom onset. Patients with SSSI were selected for this analysis. SSSI was defined as a single DWI lesion of ≤2.0 cm in size at its largest dimension in the perforator territory of the middle cerebral artery and basilar artery (infarctions in the paramedian pontine area) (10), SSSI with stenosis of any degree of the parent artery (middle cerebral artery and basilar artery) was regarded as a SSSI + PAD and SSSI without stenosis of the parent artery as SSSI – PAD.

Image Analysis/Interpretation

The method of the imaging subgroup analysis has been described before (19, 20). Briefly, all patients in the imaging subgroup study of the CHANCE trial underwent conventional MRI of brain

A

B

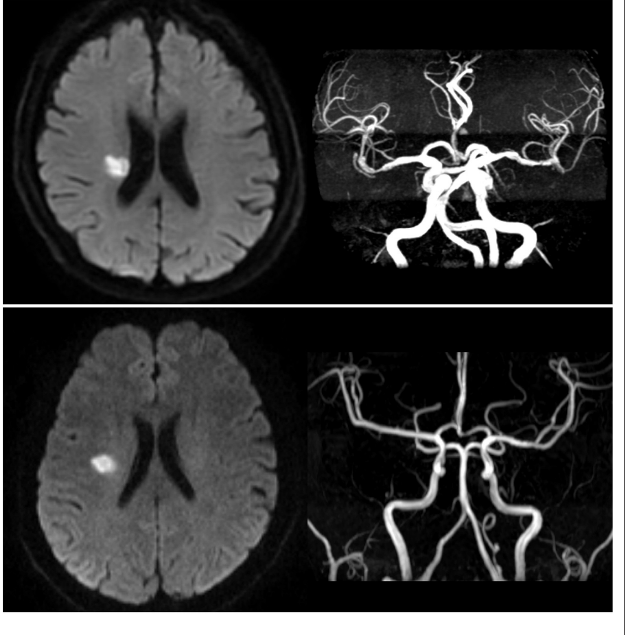

FIGURE 1 | Different types of single small subcortical infarction. **(A)** Single small subcortical infarction associated with parental artery disease (SSSI + PAD); **(B)** Single small subcortical infarction without parental artery disease (SSSI – PAD).

Abbreviations: SSSI, single small subcortical infarction; PAD, parent artery disease; CHANCE, Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Events; IS, ischemic stroke; SPS3, Secondary Prevention of Small Subcortical Strokes; TIA, transient ischemic attack; GUSTO, Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries; DWI, diffusion-weighted imaging; HR, hazard ratio; CI, confidence interval.

TABLE 1 | Baseline characteristics of the patients with SSSI + PAD and SSSI – PAD.

Characteristics	SSSI+PAD			SSSI – PAD			
	Dual antiplatelet, *n* (%)	Mono antiplatelet, *n* (%)	*P*- Value	Dual antiplatelet, *n* (%)	Mono antiplatelet, *n* (%)	*P*- Value	*P*- Value*
Patients	46 (43.8)	59 (56.2)		124 (53.2)	109 (46.9)		
Age(years)	63.6 ± 9.4	64.8 ± 9.8	0.46	61.5 ± 10.1	57.5 ± 10.1	0.003	<0.001
Male	26 (56.5)	29 (49.2)	0.56	80 (64.5)	83 (76.1)	0.06	0.002
Systolic blood pressure(mmHg)	157.8 ± 24.5	167.4 ± 27.3	0.12	157.1 ± 21.4	155.6 ± 22.8	0.54	0.04
Diastolic blood pressure (mmHg)	91.4 ± 14.0	92.7 ± 12.7	0.33	91.1 ± 13.6	92.7 ± 14.2	0.40	1.00
Body Mass Index (kg/m^2)	25.1 ± 2.8	24.4 ± 3.2	0.11	24.3 ± 3.1	24.7 ± 3.2	0.36	0.33
Previous history							
Ischemic stroke	13 (28.3)	7 (11.9)	0.05	15 (12.1)	15 (13.8)	0.84	0.14
TIA	0	2 (3.4)	0.50	1 (0.8)	1 (0.9)	1.00	0.59
Myocardial infarction	1 (2.2)	0	0.44	4 (3.2)	1 (0.9)	0.37	0.67
Angina	0	2 (3.4)	0.50	3 (2.4)	0	0.25	0.65
Cardiac dysfunction	2 (4.3)	0	0.19	1 (0.8)	0	1.00	0.23
Arrhythmia	2 (4.3)	0	0.19	1 (0.8)	3 (2.8)	0.34	1.00
Valvular heart disease	0	0	NA	0	1 (0.9)	0.47	1.00
Hypertension	33 (71.7)	42 (71.2)	1.00	72 (58.1)	69 (63.3)	0.42	0.07
Diabetes mellitus	13 (28.3)	12 (20.3)	0.37	25 (20.2)	15 (13.8)	0.23	0.18
Hyperlipidemia	5 (10.9)	6 (10.2)	1.00	15 (12.1)	9 (8.3)	0.39	1.00
Smoking	20 (43.5)	12 (20.3)	0.02	57 (46.0)	58 (53.2)	0.30	0.001
Time to randomization (hours)	12.4 ± 7.0	13.1 ± 6.5	0.61	13.3 ± 6.4	15.2 ± 6.5	0.02	0.07
Time to randomization							
<12 h	25 (54.3)	28 (47.5)	0.56	58 (46.8)	38 (34.9)	0.08	0.12
Medications							
Antihypertensive	22 (50.0)	25 (42.4)	0.55	56 (45.9)	50 (46.3)	1.00	1.00
Antidiabetic	8 (18.2)	9 (15.3)	0.79	19 (15.6)	11 (10.2)	0.25	0.40
Lipid-lowering	19 (43.2)	31 (52.5)	0.43	68 (55.7)	61 (56.5)	1.00	0.23

SSSI + PAD, Single small subcortical infarction with parental arterial disease; SSSI – PAD, Single small subcortical infarction without parental arterial disease; TIA, Transient Ischemic Attack.
P-values for comparisons between patients with and without PAD.

and three-dimensional (3D) time-of-flight magnetic resonance angiography (MRA) with a 3.0 or 1.5 T MR scanner. Other MR sequences included T1/T2-weighted imaging and diffusion-weighted imaging (DWI). All MRI/MRA images were stored in digital format and read centrally by two readers who were blinded to the subjects' clinical information or outcomes. In cases of discrepancy, the final diagnosis was reached by consensus. We assessed the following arterial segments: middle cerebral artery (M1/M2) and basilar artery, degree of intracranial stenosis on MRA was calculated by using the published method described in the Warfarin-Aspirin Symptomatic Intracranial Disease Study (21). The status of the artery was categorized as normal or PAD. Absence of distal filling on MRA would be regarded as occlusion. In this study, stenosis of any degree of PAD was regarded as a significant cause of SSSI as described in previous study (10). Patients with SSSI were divided into SSSI + PAD or SSSI – PAD groups (**Figure 1**).

Statistical Analysis

We compared the baseline characteristics of patients with SSSI + PAD or SSSI – PAD by using chi-square tests and independent sample *t* tests for categorical and continuous variables, respectively. In addition, the baseline characteristics of patients with SSSI + PAD or SSSI-PAD on the dual antiplatelet therapy or mono antiplatelet therapy were also compared. The rates of primary and secondary efficacy and safety outcomes at 90 days were compared between patients on different treatments (dual antiplatelet therapy, or mono antiplatelet therapy) with SSSI + PAD or SSSI – PAD by using chi-square tests.

Cox proportional hazards models or logistic regression models were performed with different treatments as the covariates, to obtain the hazard ratios (HR) or odds ratios (OR) and two-sided 95% confidence intervals (CI) of different treatments for the primary efficacy outcome of any stroke and the safety outcome of any bleeding, regardless of the presence of PAD. In the model, we had adjusted for age, sex, systolic blood pressure, previous history of ischemic stroke, previous history of smoking, and time to randomization. Cox proportional hazards models or logistic regression models were also performed with the treatments (clopidogrel plus aspirin or placebo plus aspirin), the presence of PAD, and the treatment by presence of PAD interaction as covariates, to test the interaction between

TABLE 2 | Efficacy and safety outcomes of the patients with SSSI + PAD and SSSI – PAD.

Outcomes	SSSI + PAD				SSSI – PAD				
	Mono antiplatelet, *n* (%)	Dual antiplatelet, *n* (%)	HR/OR (95% CI) *	*P*-Value*	Mono antiplatelet, *n* (%)	Dual antiplatelet, *n* (%)	HR/OR (95% CI)*	*P*-Value*	*P*-Value‡
Efficacy outcomes									
Primary efficacy outcome, stroke	8 (13.6)	5 (10.9)	0.84 (0.25–2.75)	0.77	8 (7.3)	11 (8.9)	1.03 (0.40–2.68)	0.95	0.83
Secondary efficacyOutcome†									
Ischemic stroke	8 (13.6)	5 (10.9)	0.84 (0.25–2.75)	0.77	8 (7.3)	11 (8.9)	1.03 (0.40–2.68)	0.95	0.83
Hemorrhagic stroke	0	0	NA	NA	0	0	NA	NA	NA
Myocardial infarction	0	0	NA	NA	0	0	NA	NA	NA
Vascular death	0	0	NA	NA	0	0	NA	NA	NA
Death from any cause	0	0	NA	NA	0	0	NA	NA	NA
TIA	1 (1.7)	0	NA	NA	2 (1.8)	0	NA	NA	NA
Disabling/fatal stroke	9 (15.3)	7 (15.9)	1.21 (0.37–3.97)	0.75	9 (8.3)	11 (9.0)	1.02 (0.39–2.72)	0.96	0.96
Safety outcomes									
Bleeding, according to GUSTO									
Severe Bleeding	0	1 (2.2)	NA	NA	0	1 (0.8)	NA	NA	0.99
Moderate Bleeding	0	0	NA	NA	0	0	NA	NA	NA
Mild Bleeding	0	0	NA	NA	0	0	NA	NA	NA
Any bleeding	1 (1.7)	3 (6.5)	4.00 (0.34–46.82)	0.27	3 (2.8)	2 (1.6)	0.33 (0.05–2.24)	0.25	0.13

CI, confidence interval; HR, hazard ratio; OR, odds ratio; GUSTO, Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries criteria; SSSI + PAD, Single small subcortical infarction with parental arterial disease; SSSI – PAD, Single small subcortical infarction without parental arterial disease.

* *Adjusted for age, male, systolic blood pressure, previous history of ischemic stroke, smoking and time to randomization.*

† *Secondary efficacy outcome: new clinical vascular events including ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death.*

‡ *P-values for interaction of treatment by presence of PAD.*

the differential effects of dual vs. mono antiplatelet therapies on the primary efficacy outcome (any stroke) and safety outcomes among patients with and without PAD. The time to the primary efficacy outcome event for each group was presented by the Kaplan-Meier curves. All tests were two-sided, and a *P*-value <0.05 was considered to be statistically significant. All statistical analyses were performed with the use of SAS software, version 9.0 (SAS Institute).

RESULTS

Between October 2009 and July 2012, a total of 5,170 patients with acute minor stroke or high-risk TIA were enrolled in the CHANCE trial. Of those, 1,089 patients at 45 centers undergoing MR examinations at baseline with all the sequences as required were included in this subgroup analysis. Compared to patients not in the imaging subgroup study, patients in the imaging subgroup study were older, more likely to have higher systolic blood pressure, lower body mass index, longer time to be randomized, higher baseline ABCD2 score for qualifying TIA, minor stroke as the qualifying event and less likely to have prior history of IS (**Supplementary Table 1**).

In the imaging subgroup, 338 with SSSI were recruited in the final analysis of this study. Among them 105 patients had SSSI + PAD and 233 had SSSI – PAD. Patients with SSSI + PAD were older (64.3 vs. 59.6, $P < 0.001$) and more likely to be female (47.6 vs. 30.0%, $P = 0.002$). They had higher systolic blood pressure (163.2 vs. 156.4, $P = 0.04$), as compared with those with SSSI – PAD (**Table 1**). In patients with SSSI + PAD, more in the dual antiplatelet therapy group had a prior history of ischemic stroke (28.3 vs. 11.9%, $P = 0.05$) and were smokers (43.5 vs. 20.3%, $P = 0.02$), as compared to those in the mono antiplatelet therapy group (**Table 1**). In patients with SSSI – PAD, patients in the dual antiplatelet therapy group were older (61.5 vs. 57.5, $P = 0.003$), shorter time to be randomized (13.3 vs. 15.2, $P = 0.02$) than those in the mono antiplatelet therapy group (**Table 1**). Other baseline characteristics were not significantly different between the two groups.

Efficacy Outcomes

In our study, 32 of the 338 patients (9.5%) with SSSI had a primary efficacy outcome of recurrent stroke during the 90-day follow-up period (**Table 2**). **Figure 2** shows the Kaplan-Meier curves presenting the time to event for the primary efficacy outcome in different groups. The addition of clopidogrel to aspirin did not significantly reduce stroke recurrence than aspirin alone among patients with SSSI + PAD (adjusted HR 0.84; 95% CI, 0.25–2.75; $P = 0.77$) and those with SSSI – PAD (adjusted HR

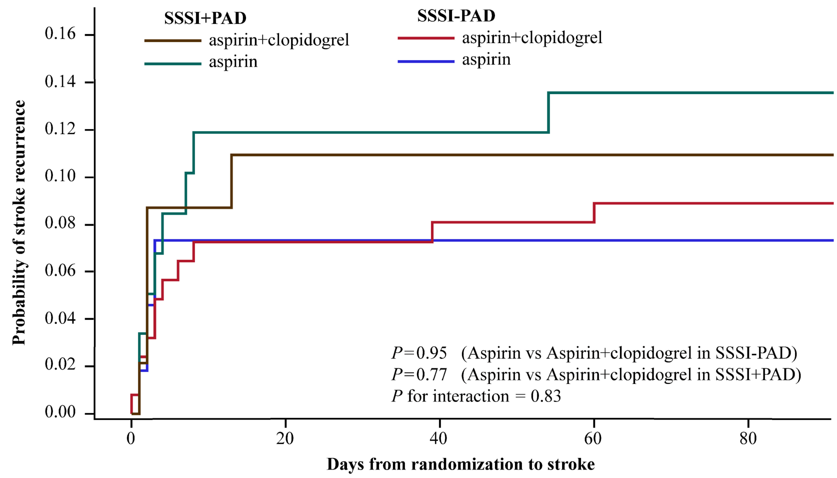

FIGURE 2 | Kaplan-Meier curves for the primary efficacy outcome of any stroke. Kaplan-Meier curves showing the time to the primary efficacy outcome event (any stroke) in patients with SSSI + PAD and SSSI – PAD, treated with clopidogrel plus aspirin, or placebo plus aspirin. SSSI, single small subcortical infarction; PAD, parental arterial disease.

1.03; 95% CI, 0.40–2.68; *P* = 0.95) (interaction *P* = 0.83; **Table 2**). In patients with SSSI + PAD, the rate of stroke recurrence in those treated with dual antiplatelet therapy and mono antiplatelet therapy was not significantly different (10.9 vs. 13.6%, *P* = 0.77) (**Table 2**). Dual antiplatelet therapy did not reduce stroke recurrence in all SSSI patients (**Supplementary Table 2**).

Safety Outcomes

Two patients in the dual therapy had severe bleeding events: one in SSSI + PAD group and one in SSSI – PAD group. The adjusted HR for the clopidogrel plus aspirin vs. aspirin alone on any bleeding event was 4.00 (95% CI, 0.34–46.82) in patients with SSSI + PAD and 0.33 (95% CI, 0.05–2.24) in patients with SSSI – PAD, respectively. No statistically significant evidence for the interaction between the types of SSSI and treatment allocation on any bleeding event (interaction *P* = 0.16; **Table 2**). Dual antiplatelet therapy did not increase the risk of any bleeding event in all SSSI patients (**Supplementary Table 2**).

DISCUSSION

We found that the combination of clopidogrel with aspirin did not reduce stroke recurrence in patients with SSSI regardless of PAD in the CHANCE trial. To our knowledge, the current subgroup analysis was the first to explore the efficiency of short-term (21 days) dual antiplatelet therapy in patients with different types of SSSI.

A few clinical trials had assessed the role of combining clopidogrel and aspirin for non-cardioembolic IS prevention (12, 14, 15, 22). The SPS3 trial was similar to this analysis, and it has concluded that dual antiplatelet therapy did not significantly reduce the risk of recurrent stroke but did significantly increase the risk of bleeding and death among patients with recent small subcortical infarctions compared to those on mono antiplatelet therapy (12). Similarly, our subgroup analysis indicated that dual antiplatelet therapy did not significantly reduce the risk of recurrent stroke in those with small subcortical infarctions. As for the risk of hemorrhage, unlike our result, the SPS3 trial found that the risk of major hemorrhage was almost doubled among those on dual antiplatelet therapy. One possible explanation would be the duration of dual antiplatelet therapy. Patients in the CHANCE trial were on dual antiplatelet therapy for 21 days while the mean duration of such treatment was 3.4 years in the SPS3 trial. Previous studies have showed that the risk of bleeding was low if the treatment was within 21 days, but increased if treated long-term (23–25). Other studies also indicated that short-term (7 days) dual antiplatelet therapy did not increase the risk of hemorrhage in patients with large artery atherosclerotic IS (14–16).

SSSI in penetrating arterial territory could be caused by the plaque from the parental artery blocking the orifice of penetrators (SSSI + PAD) or lipohyalinosis of distal small arteries (26–29). Therefore, SSSI + PAD was classified as large artery atherosclerosis instead of small artery disease in a new classification system of ischemic stroke (5). Previous trials have indicated that dual antiplatelet therapy could reduce microembolic signals in patients with predominantly intracranial symptomatic arterial stenosis (15) or carotid stenosis (14). Results from these trials supported the hypothesis that dual antiplatelet therapy was effective in treating large artery atherosclerosis stroke. In spite of the possibility of a greater effect in SSSI + PAD patients with dual antiplatelet therapy, non-significant difference was observed in our study. The fact that patients with SSSI + PAD in our study did not have significantly more indicators of atherosclerosis than those with SSSI – PAD may be one underlying cause. In addition, the number of patients in this subgroup analysis probably was underpowered to detect any significant difference between the effects of dual vs. mono-antiplatelet therapies.

There were several limitations in our study. First, only approximately 20.0% of patients in the CHANCE trial were analyzed in imaging subgroup analysis, and there were small numbers of outcome events, especially for the safety outcomes of bleeding events. It might indicate potential selection bias of the current study, considering the fact that we only included cases from 45 of 114 participating centers providing MRIs. Therefore, the current study had limited power to detect heterogeneities of the efficiency and safety of dual vs. mono antiplatelet therapies among patients with and without PAD. Secondly, all patients with SSSI in the CHANCE trial had minor stroke (National Institute of Health stroke scale score ≤3), so the extrapolation of findings from CHANCE to other populations should be made with caution. Third, we could not completely rule out the possibility that MRA lesions were due to a partial embolic occlusion as we did not exclude patients with stenosis of the ipsilateral carotid artery or vertebral artery. However, the possibility of embolic occlusion should be relatively low in our study considering embolic MCA occlusion rarely causes SSSI if infarcts are assessed with DWI according to previous studies (27, 30), and extracranial large-artery stenosis is less common in Chinese patients (31). Fourth, in light of the small sample size in this analysis, patients with SSSI + PAD were not additionally classified by the degree of artery stenosis. Future large-scale studies are needed.

CONCLUSION

Our results support the hypothesis that dual antiplatelet therapy, initiated early after ictus and lasting for a short period, does not reduce the risk of any stroke among patients with SSSI regardless of PAD. Studies on other populations with large sample size and implying HRMRI are needed in the future to verify our findings further in patients with different types of SSSI.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of Beijing Tiantan Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GW and XY interpreted the data and drafted the manuscript. JJ, XZ, LL, CW, and XM acquired the data and revised the manuscript. AW analyzed the data. DW revised the manuscript. YoW and YiW designed the research and handled funding and supervision. All authors have read and agreed on the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.631220/full#supplementary-material

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The Role of Aspirin in the Management of Intracranial Aneurysms: A Systematic Review and Meta-Analyses

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Objective: To evaluate the association between aspirin use and the risks of unruptured intracranial aneurysm (UIA) growth and aneurysmal subarachnoid hemorrhage (aSAH).

Methods: We searched PubMed and Scopus from inception to 1 September 2020. Studies evaluating the associations between aspirin prescription and the risk of UIA growth or the risk of aSAH were included. The study only included patients with intracranial aneurysms. We assessed the quality of included studies using the Newcastle-Ottawa scale. Random-effects meta-analysis was conducted to pool the estimates of effect size quantitatively. Sensitivity analyses using the leave-one-out strategy were performed to identify any potential source of heterogeneity.

Results: After a review of 2,226 citations, five cohort studies, two case-control studies, and one nested case-control study involving 8,898 participants were included. Pooled analyses showed that aspirin use, regardless of frequency and duration, was associated with a statistically significantly lower risk of UIA growth (OR 0.25, 95% CI 0.11–0.54; $I^2 = 0.0\%$, $p = 0.604$) and aSAH (OR, 0.37, 95% CI, 0.23–0.58; $I^2 = 79.3\%$, $p = 0.001$) in patients presented with intracranial aneurysms. The results did not significantly change in sensitivity analyses.

Conclusions: Summarizing available evidence in the literature, our findings indicate that aspirin use, regardless of frequency and duration, was associated with a statistically significantly lower risk of UIA growth and aSAH in patients with UIA. Well-designed and large-scale clinical trials are needed to help define the role of aspirin as a protective pharmaceutical for UIAs.

Keywords: aspirin, intracranial aneuryms, aneurysmal subarachnoid hemorrhage, prevention, meta-analysis

INTRODUCTION

According to global statistics, it is estimated that 3% of the adult population has an unruptured intracranial aneurysm (UIA) (1). With the development of non-invasive imaging techniques, an increasing number of UIAs are being detected (2). Despite the further expansion of endovascular techniques and surgical clipping in recent years, the incidence of aneurysmal subarachnoid hemorrhage (aSAH) is relatively unchanged worldwide (3). Small aneurysms (<7 mm) are often left untreated because these patients cannot benefit from existing treatments, and the risk of aneurysm rupture does not outweigh the risk of morbidity and mortality from treatment complications for these aneurysms. Due to the non-negligible rate of aneurysm growth, regular follow-up with imaging surveillance to assess change in size and morphology is indicated (4–6). However, the continuous growth of an intracranial aneurysm results in subarachnoid hemorrhage (SAH), which has a mortality of 35%, and leads to serval serious complications (7). Thus, there is an urgent need for a non-invasive pharmaceutical treatment that can mitigate the risk of UIA growth.

Recently, accumulative evidence has suggested that inflammation plays a critical role in the structural deterioration of the IA wall and its subsequent rupture (8). Several observational studies have linked a representative non-steroidal anti-inflammatory drug-aspirin use with a slower rate of IA growth and lower risk of aSAH (4, 9–15). Aspirin has been widely prescribed as a standard secondary preventative agent in patients with risks of cardio- and cerebrovascular diseases. If aspirin is proved to have a beneficial effect on the risk of UIA growth with an acceptable safety profile, it could be a promising treatment option for this indication. As such, we conducted this systematic review and meta-analysis including patients with intracranial aneurysms to evaluate the association between aspirin use and risk of UIA growth and aSAH.

METHODS

Search Strategy

We conducted this systematic review and meta-analysis following the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines 2009 (16). This systematic review and meta-analysis was not registered in the PROSPERO database. We thoroughly searched PubMed and Scopus from inception to 1 September 2020. A combination of search terms related to aspirin use (i.e., acetylsalicylic acid,) and outcomes of interest (i.e., occurrence of aSAH, growth of UIA) were used in the search strategy. We also searched the references of the included articles for further information. The details of the search strategy for each of the databases are included in **Supplementary Materials**.

Inclusion Criteria

Two collaborators (SY. and LX) individually screened the studies from two databases for eligibility according to predefined selection criteria: (i) the research design was cohort, case-control, or cross-sectional study; (ii) the study population was patients with UIAs and aspirin was the exposure factor; (iii) the primary outcome contained the prevalence of UIA growth or aSAH; and (iv) the study reported the odds ratio (OR) and corresponding 95% confidence intervals (CIs) (or OR and 95%CI can be manually derived from the study). Reviews, animal studies, clinical trials, case reports, commentaries were excluded. Disagreements were solved in a discussion with a senior author (XY.).

Data Extraction

Two investigators attentively screened the titles and abstracts of articles and excluded irrelevant studies after duplicates were removed. After the first-round review, the same investigators retrieved full reports of those potentially eligible studies for details independently and then included studies that met the inclusion criteria. The disagreement was resolved in discussions with a third reviewer.

Data were extracted from retrieved articles by two reviewers independently. Details on the name of the first author, year of publication, region, study design, age and gender ratio of participants, exposures, primary outcomes, controls, OR with 95% Cis, and covariates adjusted rates, if available, were recorded.

Quality Appraisal

We appraised included studies using the Newcastle-Ottawa Scale 10, which is a nine-point scoring system used to assess the quality of non-randomized studies included in a systematic review/meta-analysis. A high-quality study was defined as a study with at least seven points. All items were independently assessed by two investigators with disagreements resolved by group discussion.

Statistical Analysis

We preferred to pool adjusted ORs from the primary studies; otherwise, we used the unadjusted estimates. A random-effects model was used to pool the effect estimates and I^2 statistic was used to evaluate heterogeneity (0–100%). We considered $I^2 < 50\%$ as low heterogeneity, I^2 of 50–75% as moderate heterogeneity, and $I^2 > 75\%$ as statistically high heterogeneity. We performed sensitivity analyses using a "leave-one-out" strategy to clarify the potential sources of the heterogeneity between included studies which may result from differences in the study population, intervention, or comparators. Also, we planned to assess for publication bias by the Egger test and funnel plots. All analyses were conducted in Stata version 11.

RESULTS

Literature Search

Figure 1 displays the flow chart of our study. We identified 2,226 citations from PubMed and Scopus. Eight studies met the inclusion criteria and provided data with 8,898 distinct participants: one prospective cohort study reported associations between aspirin use and UIA growth/rupture; four retrospective studies of either a prospectively maintained database, a patient cohort, or a consecutive series, indicated a negative relationship between aspirin use and UIA growth or aSAH; two case-control studies and 1 nested case-control study discussed the relationship between aspirin use and risk of aSAH. **Table 1** illustrates the detailed characteristics of the included studies, whose quality was carefully assessed by the Newcastle-Ottawa Scale (see **Table 2**).

Outcome Measure

Aspirin Use and the Risk of UIA Growth

Three studies reported associations between aspirin use and UIA growth. Although Serrone et al. identified a relatively lower risk in aspirin users (OR 0.72, 95% CI 0.29–1.81),

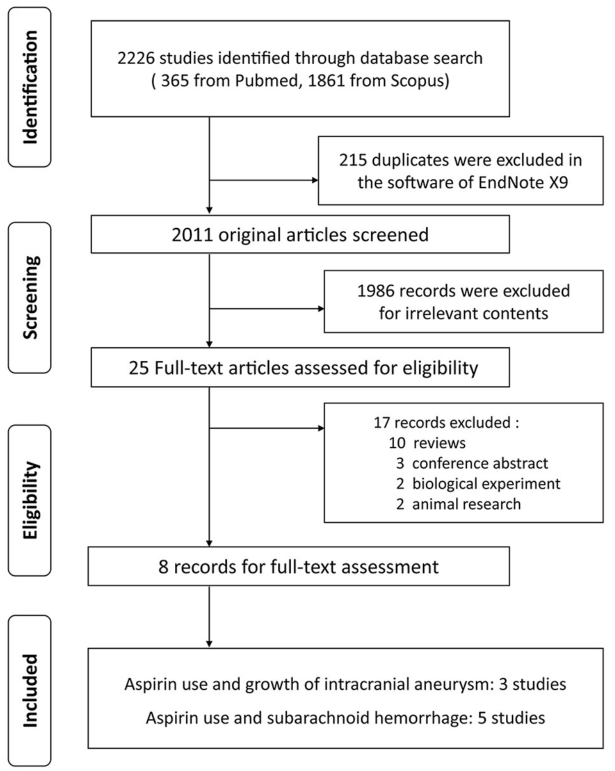

FIGURE 1 | Study screening flowchart.

their primary outcome was UIA growth or *de novo* aneurysm formation (14). Thus, we excluded it from the pooled analyses. Combining findings from the other two studies suggested that aspirin use, regardless of frequency and duration, was associated with a significantly lower risk of UIA growth (OR 0.25, 95% CI 0.11–0.54) (**Figure 2**). No significant heterogeneity was observed ($p = 0.604$).

Aspirin Use and the Risk of aSAH in Patients With UIA

Five studies reported on the association between aspirin use and risk of aSAH in patients with UIA. A meta-analysis was conducted to pool estimates of aspirin use and the risk of aSAH in UIA patients, resulting in an OR of 0.37 (95% CI, 0.23–0.58) (**Figure 3**). Significant heterogeneity was tested out in the included studies ($p = 0.001$). We then conducted a sensitivity analysis using a leave-one-out strategy. **Figure 4** showed the corresponding pooled ORs when one study was excluded from the final analysis. The results remained stable when any specific study was excluded from the pooled analysis, indicating that aspirin use was associated with a lower risk of aSAH in patients with UIA despite the high heterogeneity in studies.

DISCUSSION

In the present systematic review and meta-analysis, we summarized all available epidemiological evidence, using data from 8,898 participants involving 581 cases in the aspirin users to help clarify the association between aspirin use and UIA growth or aSAH in UIA patients. Our results showed that aspirin use, regardless of frequency and duration, was associated with a statistically significant decreased risk of UIA growth (OR 0.25, 95% CI 0.11–0.54; $I^2 = 0.0\%$, $p = 0.604$) and a significantly lower risk of aSAH (OR, 0.37, 95% CI, 0.23–0.58; $I^2 = 79.3\%$, $p = 0.001$) in patients with UIAs. The results of this study suggest that aspirin could play a role in reducing the risk of intracranial aneurysm expansion and the risk of aSAH, and aspirin could be a potential drug to treat intracranial aneurysms.

Two previous meta-analyses have discussed the effect of aspirin prescription on the risk of aSAH (6, 19). Both meta-analyses found no significant difference between aspirin users and non-aspirin users regarding the risk of aSAH (OR, 1.00; 95% CI, 0.81–1.24, $p = 0.99$, and OR, 0.981,95% CI, 0.773–1.312, $p = 0.897$). However, neither of the two meta-analyses focused on the risk of aSAH in the specific patient group with intracranial aneurysms, which may attenuate the possible protective effect of aspirin on IA rupture and aSAH in UIA patients. Moreover, better concomitant risk factor management in the UIA patients, particularly blood pressure control, might contribute to the lower risk of UIA progression. Besides, Phan et al. reported a significant association between short-term use of aspirin (<3 months) and risk of aSAH (OR, 1.61; 95% CI, 1.20–2.18, $p = 0.002$) (6). Qian et al. also reported that short-term use of aspirin (<3 months) was associated with an elevated risk of aSAH (OR, 1.697, 95% CI, 1.175–2.452, $p = 0.005$) (19). They concluded that when prescribing aspirin for prophylactic use, particularly with known UIAs, its inherent bleeding risks should be taken into consideration, especially in the short term. Several population-based studies have explored the association between antiplatelet therapy and SAH, reaching conflicting results (20, 21). Recently, Weng et al. provided Class III evidence in a prospective, multicenter cohort that for patients harboring UIAs <7 mm with ischemic cerebrovascular disease, aspirin does not increase the risk of aneurysm rupture (17). Together with our findings, we believe that the benefit of aspirin uses in this specific population outweighs the possibly increased risk of aSAH.

Both animal experiments and human clinical studies indicate that vascular remodeling and inflammatory cascades are crucial in the formation, progression, and rupture of IAs (22). Abnormal wall shear stress-activated the PGE2 (prostaglandin E2) -EP2 (prostaglandin E receptor 2) pathway in endothelial cells (ECs) at the early stage of cerebral aneurysm formation (23, 24). Subsequently, vascular smooth muscle cell apoptosis and migration, accompanied by inflammatory cell infiltration, resulted in degradation of the vascular wall, leading to the progression, and eventual rupture of IAs (22). Hasan et al. found in a small patient group that cyclooxygenase-2 (COX-2) and microsomal prostaglandin E2 synthase-1 (mPGES-1) are expressed in human cerebral aneurysms and expression increases in ruptured aneurysms (25). Thus, drugs targeting molecules involved in the above process might have potential therapeutic effects. As a commonly used preventative agent in patients with risks of cardio- and cerebrovascular diseases, aspirin has been shown to have inhibitory effects on several

TABLE 1 | Characteristics of included studies in the systematic review.

Study Authors and Published Year (location)	Study design	Inclusion criteria for participants	Definition of aspirin users	Number of Cases in the exposure group	Follow-up Duration, mean	Definition of outcomes	Adjusted estimate, (95% CI)/other outcomes	Adjustment of covariates
Weng et al. (17)	Prospective cohort study	Patients with UIAs <7 mm and concurrent ischemic cerebrovascular diseases between Jan 2016 and Dec 2019. (*n* = 272)	**Aspirin users** were defined as those who reported aspirin use at least 3× per week, including standard- and low-dose aspirin. **Non-aspirin users** were those who used no aspirin.	113	19.6 months	**The primary outcome**: Aneurysm growth, which was defined as [1] growth ≥1.0 mm in at least 1 direction by identical imaging modalities, [2] growth ≥0.5 mm in 2 directions by identical imaging modalities, and [3] an indisputable change in aneurysm shape. **The secondary outcome:** UIA rupture. The diagnosis of aneurysm rupture was confirmed by preoperative CT, MR imaging, cerebrospinal fluid analysis, or a neurosurgeon during operation.	**The primary outcome**: **HR, 0.29 (0.11–0.77)** The cumulative annual growth rates were as high as 40.0 and 53.3 per 100 person-years in the high-risk patients (>1 risk factor) with and without aspirin, respectively. **The secondary outcome**: No aneurysm rupture	Age, female sex, hyperlipidemia, pretransient ischemic attack, or ischemic stroke
Zanaty et al. (9) (Japan)	A retrospective review of a prospectively maintained database	[1] Patients harbored multiple saccular IAs; [2] At least one primary aneurysm was treated with coiling, stent-assisted coiling, flow diversion, or microsurgical clipping;[3] The remaining aneurysms were ≤5 mm in size and observed for growth; and [4] At least 5 years of follow-up from the initial treatment was available. (*n* = 146)	**Aspirin users** were defined as those who reported aspirin use ≥81 mg daily. **Non-aspirin users** were those who used no aspirin.	69	More than 5 years	**The primary outcome**: the interval growth of any remaining untreated aneurysms that later required treatment. Growth was defined as an increase in the size of the aneurysm ≥1 mm. All aneurysms that demonstrated growth underwent treatment regardless of size.	**The primary outcome**: **OR, 0.19 (0.05–0.63)**	Patient sex and age, aneurysm size and location, rupture status of the designated primary aneurysm at the initial encounter, hypertension, diabetes mellitus, hypercholesterolemia, use of other anticoagulant or antiplatelet medication, family history of IAs, drug abuse, polycystic kidney disease.
Serrone et al. (14) (United States)	A retrospective review of a patient cohort	Patients are seen in the clinic with the diagnosis of an untreated UIA and at least 1 follow-up clinic visit or consultation. (*n* = 192)	**Aspirin users** were defined as those who reported aspirin use. **Non-aspirin users** were those who used no aspirin.	120	11.5	**The primary outcome:** Aneurysm growth or de novo aneurysm formation	**The primary outcome**: **OR, 0.72 (0.29–1.81)**	NA
Gross et al. (11) (United States)	A retrospective review of a consecutive series	Patients with at least one cerebral aneurysm seen by the neurosurgical service during the study period. (*n* = 717)	**Aspirin users** were defined as those who reported aspirin use (81 or 325 mg). **Non-aspirin users** were those who used no aspirin.	32	7 years	**The primary outcome:** aneurysmal subarachnoid hemorrhage	**The primary outcome**: **OR, 0.58 (0.38–0.90)**	NA

(Continued)

TABLE 1 | Continued

Study Authors and Published Year (location)	Study design	Inclusion criteria for participants	Definition of aspirin users	Number of Cases in the exposure group	Follow-up Duration, mean	Definition of outcomes	Adjusted estimate, (95% CI)/other outcomes	Adjustment of covariates
Can et al. (12) (United States)	Case-control study	Patients who were diagnosed with an intracranial aneurysm between 1990 and 2016 (*n* = 4,619).	**Aspirin users** were defined as those who reported aspirin use. **Non-aspirin users** were those who used no aspirin.	99	NA	**The primary outcome:** aneurysmal subarachnoid hemorrhage	**The primary outcome**: **OR, 0.60 (0.45–0.80)**	Age, sex, and race, and comorbid conditions, including hypertension, coronary artery disease, myocardial infarction, and atrial fibrillation, antihypertensive medication use, family history of aneurysms or SAH, and current tobacco and alcohol use.
Hostettler et al. (13) (United Kingdom)	Case-control study	Patients with aneurysmal SAH or unruptured aneurysm without previous SAH enrolled in the Genetic and Observational Subarachnoid Hemorrhage study (*n* = 2,334).	Aspirin use was defined by patient self-reporting or available documentation on regular intake at the time of either admission with aneurysmal SAH or of being diagnosed with an unruptured aneurysm	120	NA	**The primary outcome:** aneurysmal subarachnoid hemorrhage	**The primary outcome**: **OR, 0.28 (0.20–0.40)**	Age, sex, ethnicity, smoking status, use of antihypertensive medication, hypercholesterolemia, aneurysm location, aneurysm size.
Nisson et al. (15) (United States)	Retrospective cohort study	Patients who underwent surgery for intracranial aneurysm between January 2010 and April 2013 at a tertiary academic medical center (*n* = 347).	**Aspirin users** were defined as those who reported aspirin use. **Non-aspirin users** were those who used no aspirin.	9	11.5	**The primary outcome:** aneurysmal subarachnoid hemorrhage	**The primary outcome**: **OR, 0.18 (0.09–0.39)**	NA
Hasan et al. (10) (United States)	Nested case-control study	[1] Patients must have at least one UIA, which may or may not be symptomatic. [2] Patients who have had a ruptured aneurysm at another location that was isolated, trapped, clipped, or treated through endovascular obliteration must be able to care for themselves after the aneurysmal treatment according to a follow-up evaluation at 30 days of post-treatment. (*n* = 271)	**Aspirin users** were defined as those who reported aspirin use based on questionnaires. **Non-aspirin users** were those who used no aspirin.	19	5 years	**The primary outcome:** UIA rupture. The adjudicated hemorrhage events were defined as a primary hemorrhage if either: [1] a definite or highly probable SAH of aneurysmal or unknown etiology or [2] a definite or highly probable intracranial hemorrhage determined to be of aneurysmal etiology.	**The primary outcome**: **OR, 0.27 (0.11–0.67)**	Age, sex, UIA enrollment group, participating center location, multiple aneurysm, hypertension, cardiac valvar disease, atrial fibrillation-flutter, other cardiac arrhythmias, congestive heart failure, myocardial infarction, family history of intracranial aneurysm hemorrhage, smoking, alcohol consumption, use of anticoagulants, history of aneurysms, interaction smoking and hypertension.

OR, odds ratio; CI, confidence interval; NA, not available.

TABLE 2 | Newcastle-Ottawa scale for assessing the quality of included studies.

Study design	Author, year (Pubmed ID)	Selection (Max=4)	Comparability (Max=2)	Exposure (Max=3)	Overall quality score (Max=9)
Case-control study	Can et al. (12) (30135253)	4	2	2	8
	Hostettler et al. (13) (28973585)	4	2	2	8
	Hasan et al. (18) (21980208)	4	2	2	8
Study design	**Author, year (Pubmed ID)**	**Selection (Max=4)**	**Comparability (Max=2)**	**Outcome (Max=3)**	**Overall quality score (Max=9)**
Cohort study	Weng et al. (32878566)	4	2	3	9
	Nisson et al. (15) (31857268)	4	1	3	8
	Zanaty et al. (9) (31662579)	4	2	3	9
	Serrone et al. (14) (26967775)	4	1	2	7
	Gross et al. (11) (23548847)	4	1	3	8

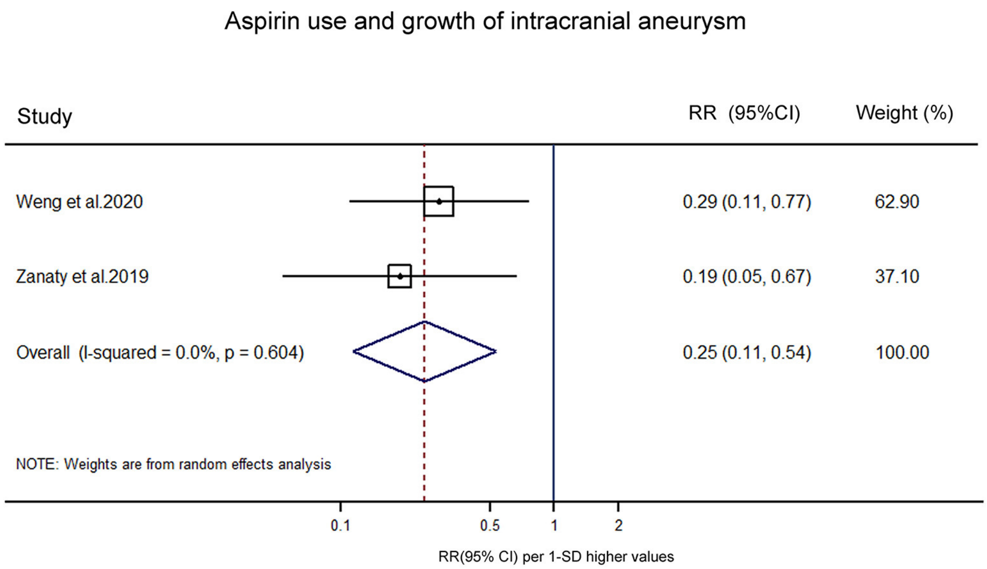

FIGURE 2 | Forest plot for an association between aspirin use and growth of intracranial aneurysm. OR, odds ratio; CI, confidence interval.

inflammatory mediators such as COX-2 and mPGES-1, making it one of the promising drugs for decreasing UIA growth and rupture (10). Several groups have proved that acetylsalicylic acid (ASA) was associated with a slower IA growth rate and lower IA rupture or aSAH rate in mice IA-induction models, suggesting the protective effect of ASA against IA rupture (8). Moreover, Hasan et al. demonstrated a decreased expression of inflammatory cells and markers such as COX-2 in a small randomized sample of patients with unruptured aneurysms who underwent microsurgical clipping after 3 months of aspirin treatment (18). More researches should be conducted to further elucidate the underlying mechanisms of this issue.

The present study was constrained by several limitations. Firstly, the number of included studies was relatively low, especially for the meta-analysis on UIA growth. Secondly, all eligible data included in the meta-analysis were extracted from observational studies and most studies were retrospective. Last but not least, heterogeneity among studies suggests that the effect of aspirin on UIA growth and rupture should be further confirmed by clinical trials. Re-analyzing existing non-randomized data using advanced statistical techniques (i.e., inverse probably of treatment weighting) could better explore this association as well.

CONCLUSION

Summarizing available evidence in the literature, our findings indicate that aspirin use, regardless of frequency and duration,

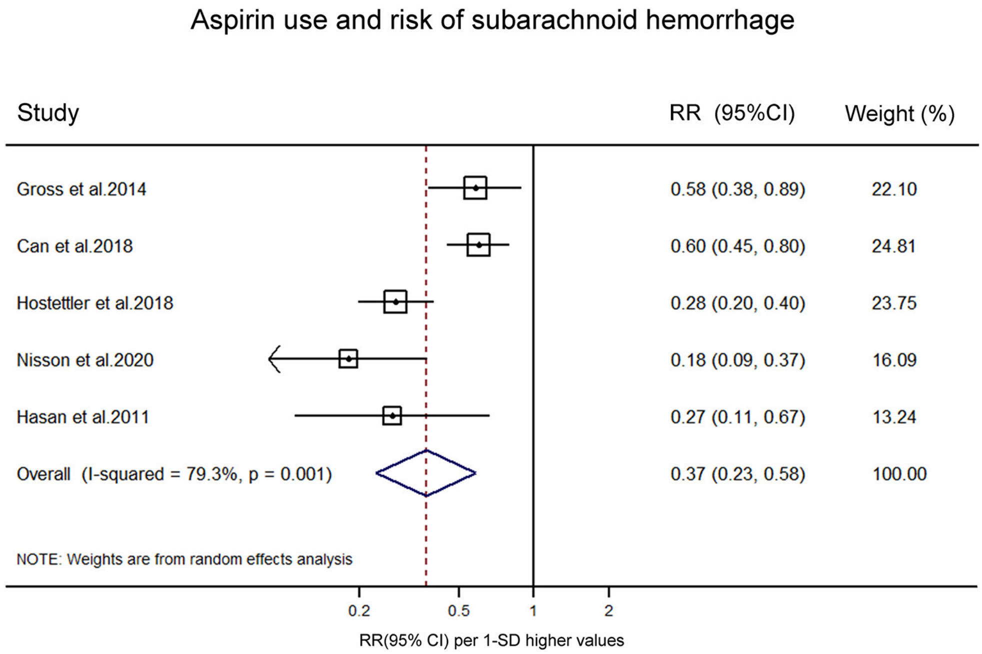

FIGURE 3 | Forest plot for an association between aspirin use and risk of subrachnoid hemorrhage. OR, odds ratio; CI, confidence interval.

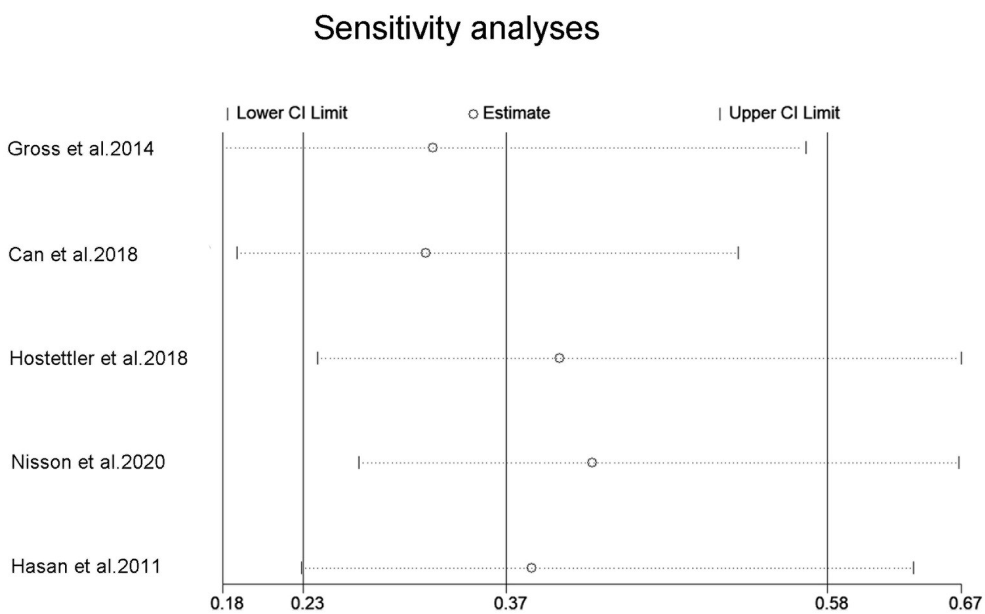

FIGURE 4 | Sensitivity analysis for an association between aspirin use and risk of subrachnoid hemorrhage. OR, odds ratio; CI, confidence interval.

was associated with a statistically significant decreased risk of UIA growth and aSAH in UIA patients. Aspirin might be a potential drug for the treatment of intracranial aneurysms. Well-designed, large-scale clinical trials are needed to help definitively define aspirin's role as a protective pharmaceutical for UIAs.

AUTHOR CONTRIBUTIONS

SY, LX, and XY contributed to the conception or design of the work and contributed to the acquisition, analysis, or interpretation of data for the work. SY and LX drafted the manuscript. TL, YW, and NX critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.646613/full#supplementary-material

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ORIGINAL RESEARCH
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Impact of Platelet Endothelial Aggregation Receptor-1 Genotypes on Long-Term Cerebrovascular Outcomes in Patients With Minor Stroke or Transient Ischemic Attack

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Background: Platelet endothelial aggregation receptor-1 (PEAR1) rs12041331 has been reported to affect agonist-stimulated platelet aggregation, but it remains unclear whether this variant plays a role in recurrent stroke. Here we assess the clinical relevance of PEAR1 rs12041331 in acute minor ischemic stroke (AMIS) and transient ischemic attack (TIA) Chinese patients treated with dual antiplatelet therapy (DAPT).

Methods: We recruited 273 consecutive minor stroke and TIA patients, and Cox proportional hazard regression was used to model the relationship between PEAR1 rs12041331 and thrombotic and bleeding events.

Results: Genotyping for PEAR1 rs12041331 showed 49 (18.0%) AA homozygotes, 129 (47.3%) GA heterozygotes, and 95 (34.7%) GG homozygotes. No association was observed between PEAR1 rs12041331 genotype and stroke or composite clinical vascular event rates (ischemic stroke, hemorrhagic stroke, TIA, myocardial infarction, or vascular death) or bleeding events regardless if individuals carried one or two copies of the A allele. Our results suggested that rs12041331 genetic polymorphism was not an important contributor to clinical events in AMIS and TIA patients in the setting of secondary prevention.

Conclusions: Our data do provide robust evidence that genetic variation in PEAR1 rs12041331 do not contribute to atherothrombotic or bleeding risk in minor stroke and TIA patients treated with DAPT.

Keywords: acute minor ischemic stroke, transient ischemic attack, PEAR1, genetic polymorphism, cerebrovascular outcomes

INTRODUCTION

Patients with acute minor ischemic stroke (AMIS) and transient ischemic attack (TIA) have a high risk of recurrent stroke and cardiovascular events (1). Current guidelines recommend dual antiplatelet therapy (DAPT) with aspirin and clopidogrel as a standard of care for patients with AMIS and TIA who can be treated within 24 h after the onset of symptoms (2, 3). However, CHANCE trial reported that up to 8.2% of patients receiving DAPT still experienced a recurrent stroke (2). Antiplatelet drug resistance was found to contribute to recurrent stroke (4, 5), which may be associated with many potential genetic and environmental factors (6). However, the correlation between genetic polymorphisms and recurrent stroke is not yet fully elaborated.

Platelet endothelial aggregation receptor-1 (PEAR1) is a platelet transmembrane tyrosine kinase receptor involved in platelet–platelet contact and platelet aggregation, which is highly expressed in platelets and endothelium (7). Studies have shown that genetic variants in the PEAR1 gene were not only associated with platelet aggregation (8, 9) but also the response to antiplatelet agents including aspirin (10) and clopidogrel (11). Rs12041331 is an intronic variant that is supposed to modify PEAR1 expression in the protein level *via* a different allele-specific DNA methylation and has been found to be correlated with platelet aggregation (12, 13). Although there has been evidence that PEAR1 rs12041331 has an effect on agonist-stimulated platelet aggregation in aspirin-treated patients (14), it is still controversial whether this genetic polymorphism in PEAR1 is associated with clinical outcomes. Xu et al. reported that PEAR1 rs12041331 plays an important role in early cardiovascular outcomes in patients undergoing percutaneous coronary intervention in a Chinese population (15). However, in a white population, Yang et al. could not replicate previous reports from experimental studies or obtained in patients suggesting that PEAR1 might be a susceptibility gene for cardiovascular complications (16). Currently, research on the relationship between PEAR1 rs12041331 and the clinical events were mainly focused on coronary heart diseases. In a retrospective, case–control study, the A allele showed a higher frequency than the G allele in the recurrent ischemic stroke group (17). However, there has been no study on the relationship between PEAR1 polymorphism and the long-term cerebrovascular events in patients with minor stroke and TIA.

To assess the clinical relevance of PEAR1 rs12041331 in Chinese AMIS and TIA patients treated with DAPT, we investigated the prevalence of PEAR1 rs12041331 genotypes and estimated its association with long-term cerebrovascular events, bleeding events, and clinical function.

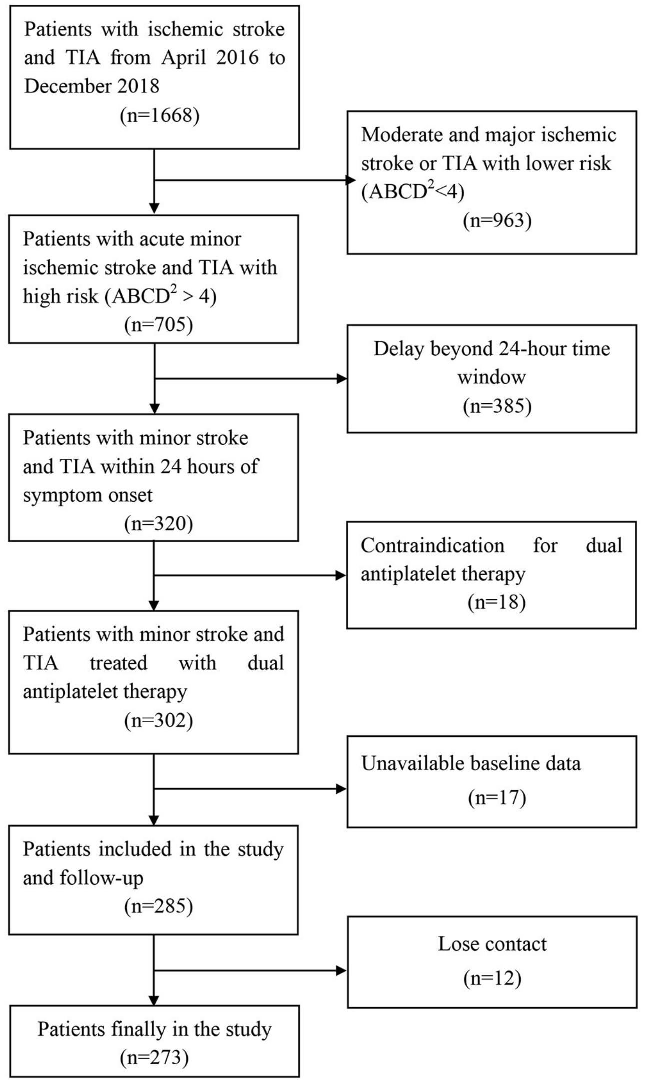

FIGURE 1 | Trial profile. TIA, transient ischemic attack.

METHOD

Study Population

We conducted a single-center cohort study based on data collected from April 2016 to December 2018 from 273 AMIS and TIA patients in the Department of Neurology of the Yangpu Hospital, Tongji University School of Medicine. The collection and genetic analysis of samples were approved by the ethics committee of Yangpu Hospital, Tongji University School of Medicine (Ethical Approval Number LL-2016-SCI-001). Informed consent has been obtained. The study enrolled patients who were at least 40 years of age and had an AMIS, with a National Institutes of Health Stroke Scale score of $\leq$3 on admission (range, 0–42, with higher scores indicating a more severe stroke), or those with a moderate to high risk of TIA according to an $ABCD^2$ stroke risk score of $\geq$4 on admission (range, 0–7, with higher scores indicating a higher risk of stroke) or $\geq$50% stenosis of cervical or intracranial vessels that could account for the presentation who could be treated with DAPT (100 mg aspirin and 75 mg clopidogrel, once daily for 21 days) within 24 h of symptom onset. Patients were excluded from the study if they had hemorrhage on baseline brain computed tomography (CT) or another pathology that could account for the neurological symptoms or had a contraindication to aspirin or clopidogrel.

Outcomes

We analyzed the relationship between the common variant PEAR1 rs12041331 and the clinically adjudicated long-term cerebrovascular events, bleeding events, and clinical function after DAPT application. The primary efficacy endpoint for this trial was a new stroke event (ischemic or hemorrhagic) that happens within 2 years. Ischemic stroke is defined as

TABLE 1 | Baseline characteristics of patients with different PEAR1 genotypes.

Characteristics	AA (*n* = 49)	GA (*n* = 129)	GG (*n* = 95)	*P*-value	AA + GA (*n* = 178)	GG (*n* = 95)	*P*-value
Age, years	65 (61–76.5)	66 (59–77)	67 (62–80)	0.815	66 (59.75–77)	67 (62–80)	0.217
Male sex, *n* (%)	34 (69.4)	88 (68.2)	69 (72.6)	0.772	122 (68.5)	69 (72.6)	0.482
Body mass index, kg/m^2	23.94 (22.48–26.76)	24.49 (22.45–25.95)	24.44 (22.60–25.95)	0.853	24.42 (22.47–26.06)	24.44 (22.60–25.95)	0.560
Diagnosis, *n* (%)				0.388			0.298
Minor stroke	46 (93.9)	115 (89.1)	82 (86.3)		161 (90.4)	82 (86.3)	
TIA	3 (6.1)	14 (10.9)	13 (13.7)		17 (9.6)	13 (13.7)	
Hypertension, *n* (%)	41 (83.7)	101 (78.3)	70 (73.7)	0.384	142 (79.8)	70 (73.7)	0.250
Diabetes mellitus, *n* (%)	22 (44.9)	51 (39.5)	48 (50.5)	0.261	73 (41)	48 (50.5)	0.132
Previous stroke, *n* (%)	5 (10.2)	13 (10.1)	23 (24.2)	0.008	18 (10.1)	23 (24.2)	0.002
Previous coronary artery disease, *n* (%)	2 (4.1)	10 (7.8)	10 (10.5)	0.398	12 (6.7)	10 (10.5)	0.274
Previous or current smoker, *n* (%)	25 (51.0)	60 (46.5)	42 (44.2)	0.74	85 (47.8)	42 (44.2)	0.576
Alcohol, *n* (%)	8 (16.3)	23 (17.8)	17 (17.9)	0.968	31 (17.4)	17 (17.9)	0.921
NIHSS	1 (0–2)	1 (0–2)	1 (0–3)	0.502	1 (0–2)	1 (0–3)	0.401
mRS	1 (0.5–2)	1 (0–2)	1 (0–2)	0.73	1 (0–2)	1 (0–2)	0.833
TOAST				0.681			0.969
Large artery atherosclerosis	23 (46.9)	49 (38.0)	40 (42.1)		72 (40.4)	40 (42.1)	
Cardioaortic embolism	0	7 (5.4)	5 (5.3)		7 (3.9)	5 (5.3)	
Small artery occlusion	24 (49.0)	63 (48.8)	45 (47.3)		87 (48.9)	45 (47.3)	
Other causes	2 (4.1)	5 (3.9)	3 (3.2)		7 (3.9)	3 (3.2)	
Undetermined causes	0	5 (3.9)	2 (2.1)		5 (2.8)	2 (2.1)	
Creatinine, μmol/L	74.73 ± 26.19	81.24 ± 34.37	82.13 ± 37.67	0.478	79.45 ± 32.39	82.13 ± 37.67	0.540
Glucose, mmol/L	7.27 ± 3.51	6.88 ± 3.05	7.65 ± 4.85	0.057	6.99 ± 3.18	7.65 ± 4.85	0.177
HDL-C, mmol/L	1.01 ± 0.24	1.03 ± 0.27	1.10 ± 0.31	0.113	1.02 ± 0.27	1.10 ± 0.31	0.057
LDL-C, mmol/L	3.20 ± 0.65	2.97 ± 0.85	3.07 ± 1.04	0.059	3.03 ± 0.81	3.07 ± 1.04	0.751
Antiplatelet drugs after 21 days				0.472			0.374
Aspirin	45	123	87		168	87	
Clopidogrel	4	6	8		10	8	

Values are presented as mean ± SD or number of patients (percentage) as appropriate. P-values represent the statistical difference of each variable by PEAR1 rs12041331 genotype. TIA, transient ischemic attack; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale; TOAST, trial of org 10,172 in acute stroke treatment; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

a sudden focal neurological dysfunction caused by vascular causes with duration ≥24 h or a neurological dysfunction due to imaging and clinical symptoms caused by bloody infarction rather than cerebral hemorrhage found by imaging examination. Hemorrhagic stroke is defined as the acute extravasation of blood into the brain parenchyma or subarachnoid space with associated neurological symptoms. A diagnosis of stroke should be confirmed by neuroimaging (CT or MRI).

The secondary efficacy endpoint was analyzed as the individual or composite outcomes of the new clinical vascular event (ischemic stroke, hemorrhagic stroke, TIA, myocardial infarction, or vascular death). Vascular death is defined as death resulting from stroke (ischemic or hemorrhagic), systemic hemorrhage, myocardial infarction, congestive heart failure, pulmonary embolism, sudden death, or arrhythmia. If multiple vascular events occurred in the same patient during the follow-up period, the composite event was counted as one person time.

A safety endpoint included intracranial hemorrhage and bleeding of any other cause. Bleeding events were classified as major bleeding (a decrease in hemoglobin level of 2 g/dl or greater within a 24-h period or leading to a transfusion of two or more units of packed red cells or requiring an additional intervention) or minor bleeding according to the International Society on Thrombosis and Hemostasis criteria (18).

The study included one visit, which was 2 years (1 month either way) after the start of DAPT. Face-to-face or telephone interviews were involved in all visits, with data collected on electronic case report forms.

Genetic Analysis

Genomic DNA was extracted from whole blood samples (empty stomach on the early morning of the day after admission) with a Lab-Aid nucleic acid (DNA) magnetic bead separation kit (Zeesan, Xiamen) according to the manufacturer's instructions. The PEAR1 rs12041331 in human whole blood genomic DNA was detected by the combination of multiplex allele-specific PCR and universal array developed by CapitalBio Technology Corporation, Ltd. Using human whole blood genomic DNA as the template, amplicons from PEAR1 gene were multiplex PCR-amplified with allele-specific PCR primers. After amplification, the reaction mixture was hybridized with specific tag probes immobilized on a microarray chip in the CapitalBio BioMixerTM II Microarray Hybridization Station (CapitalBio Corporation, Beijing, China). Hybridization was stopped by washing the slide in a wash buffer. The chips were scanned and imaged using LuxScan 10K-B Microarray Scanner (CapitalBio Corporation, Beijing, China). The detection results of polymorphic loci were obtained.

Statistical Analysis

Continuous variables were described as median (interquartile intervals, 25–75), categorical variables were expressed as frequencies and percentages, and differences of the baseline characteristics among AA, GA, and GG genotypes of PEAR1 rs12041331 were assessed by one-way ANOVA (for continuous variables) or χ^2-test (for categorical variables). Cox proportional hazard models were adopted to perform a primary analysis comparing the cumulative incidences of 2-year cerebrovascular events among patients with AA, GA, and GG genotypes of PEAR1 rs12041331. The results were presented as hazard ratio (HR) with 95% CI.

The statistical analysis was carried out using SPSS, version 21.0 (IBM Corporation, Armonk, NY, USA) and Stata, version 15.0 (Stata Corporation, College Station, TX, USA) statistical software. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics of Patients by PEAR1 Genotypes

From April 2016 to December 2018, a total of 1,668 patients were involved, of whom 320 had minor stroke and TIA within 24 h of symptom onset. A total of 18 patients had contraindication for DAPT during the treatment period, 17 patients had unavailable baseline data, and 12 patients lost contact during the follow-up (**Figure 1**). Overall, 273 patients were enrolled and contributed samples for genotyping in this study. The baseline characteristics were similar among the groups (**Table 1**). Most patients (89%) presented with minor stroke, and 11% patients presented with TIA. The median age of the patients was 66 years old, and 70% were men. The mean BMI of the patients was 24 kg/m^2. Most patients continued to use aspirin after 21-day DAPT. Genotyping for PEAR1 rs12041331 showed 49 (18.0%) AA homozygotes, 129 (47.2%) GA heterozygotes, 178 (65.2%) AA + GA heterozygotes, and 95 (34.7%) GG homozygotes. Patients with the GG homozygotes have a higher history of stroke. The distributions of age, sex, BMI, vascular risk factors, NHISS, mRS, TOAST, and laboratory data variables were not statistically different among the groups.

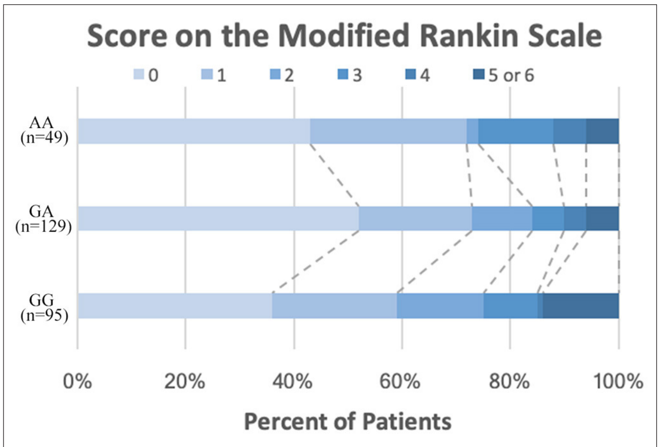

FIGURE 2 | Distribution of scores on the modified Rankin scale at 2 years. The distribution of scores for disability on the modified Rankin scale among patients by different PEAR1 rs12041331 genotype ranges from 0 to 6, with higher scores indicating more severe disability.

Efficacy Outcomes and Genotypes

All patients completed the 2-year clinical follow-up. The primary outcome (stroke) was observed in 23 of 273 patients (8.42%), and the secondary efficacy outcomes (composite clinical vascular events) occurred in 24 patients (8.79%). The median score for disability on the modified Rankin scale (mRS) at 2 years was 1 in the AA homozygotes, 0 in the GA homozygotes, and 1 in the GG homozygotes of PEAR1 rs12041331 (**Figure 2** and **Table 2**).

We did not observe any evidence of the association between PEAR1 rs12041331 genotype and cerebrovascular events in participants treated with DAPT. PEAR1 rs12041331 A allele carrier status did not result in statistically significant differences in stroke or composite clinical vascular event rates (ischemic stroke, hemorrhagic stroke, TIA, myocardial infarction, or vascular death) regardless if the individuals carried one (stroke, $P = 0.545$; composite events, $P = 0.759$; ischemic stroke, $P = 0.539$; hemorrhagic stroke, $P =$ N/A; TIA, $P =$ N/A; myocardial infarction, $P =$ N/A; vascular death, $P = 0.751$) or two (stroke, $P = 0.229$; composite events, $P = 0.334$; ischemic stroke, $P = 0.441$; hemorrhagic stroke, $P = 0.646$; TIA, $P =$ N/A; myocardial infarction, $P =$ N/A; vascular death, $P =$ N/A) copies of the A allele (**Figure 3A** and **Table 2**). We repeated these analyses between all AA/GA carriers and GG homozygotes and found

TABLE 2 | Association of PEAR1 rs12041331 with cerebrovascular events within 2 years.

Vascular events	AA (n = 49)	GA (n = 129)	GG (n = 95)	AA vs. GG		GA vs. GG		AA + GA vs. GG	
				HR 95% CI	P	HR 95% CI	P	HR 95% CI	P
Primary efficacy outcomes									
Stroke	6	11	6	2.00 (0.65–6.21)	0.229	1.36 (0.50–3.68)	0.545	1.53 (0.60–3.89)	0.368
Secondary efficacy outcomes									
Composite events	6	11	7	1.71 (0.58–5.09)	0.334	1.16 (0.45–2.99)	0.759	1.31 (0.54–3.16)	0.549
Ischemic stroke	5	11	6	1.60 (0.49–5.23)	0.441	1.37 (0.51–3.69)	0.539	1.43 (0.56–3.66)	0.455
Hemorrhagic stroke	1	0	1	1.91 (0.12–30.63)	0.646	N/A	N/A	0.53 (0.03–8.45)	0.652
TIA	0	0	1	N/A	N/A	N/A	N/A	N/A	N/A
MI	0	0	1	N/A	N/A	N/A	N/A	N/A	N/A
Vascular death	0	2	1	N/A	N/A	1.47 (0.13–16.26)	0.751	1.06 (0.10–11.72)	0.960
Death from any cause	2	7	7	0.54 (0.11–2.61)	0.447	0.74 (0.26–2.10)	0.57	0.68 (0.25–1.84)	0.451
Primary safety outcomes									
Intracranial hemorrhage	1	0	1	1.91 (0.12–30.63)	0.646	N/A	N/A	0.53 (0.03–8.45)	0.652
Any bleeding	4	6	8	0.96 (0.29–3.19)	0.947	0.55 (0.19–1.58)	0.267	0.66 (0.26–1.68)	0.386

Values are presented as number of events (percentage). HR was calculated by using Cox proportional hazards model.
TIA, transient ischemic attack; MI, myocardial infarction; N/A, not applicable.

no significant associations either (stroke, $P = 0.368$; composite events, $P = 0.549$; ischemic stroke, $P = 0.455$; hemorrhagic stroke, $P = 0.652$; TIA, P = N/A; myocardial infarction, P = N/A; vascular death, $P = 0.960$) (**Figure 3B** and **Table 2**).

Safety Outcomes and Genotypes

The safety outcome (bleeding events) occurred in 17 patients (6.23%); among them, two were with intracranial hemorrhage. The rates of safety endpoints did not differ significantly between PEAR1 rs12041331 genotypes in participants treated with DAPT. PEAR1 rs12041331 A allele carrier status did not result in statistically significant differences in bleeding events (intracranial hemorrhage and any bleeding) regardless if individuals carried one (intracranial hemorrhage, P = N/A, and any bleeding, $P = 0.267$) or two (intracranial hemorrhage, $P = 0.646$, and any bleeding, $P = 0.947$) copies of the A allele (**Table 2**). We repeated these analyses between all AA/GA carriers and GG homozygotes and found no significant associations either (intracranial hemorrhage, $P = 0.652$, and any bleeding, $P = 0.386$; **Table 2**).

DISCUSSION

Major Findings

In the present study, we evaluated the impact of PEAR1 rs12041331, a well-described genetic variant implicated in aspirin-related platelet function, on long-term cerebrovascular events, bleeding events, and clinical function in AMIS and TIA patients treated with DAPT. Unfortunately, it was observed in the current study that PEAR1 rs12041331 genotype was not associated with the atherothrombotic or bleeding events in minor stroke and TIA.

Comparison With Prior Studies

PEAR1 receptor, which is highly expressed in platelets and endothelial cells, is a critical part of platelet aggregation response toward multiple agonists, and rs12041331 is a strong genetic determinant of on-treatment platelet inhibition (7, 10, 19). However, few data are reported regarding the impact of this variant on cerebrovascular event risk in AMIS and TIA patients treated with DAPT. Investigations focused on the impact of this polymorphism were mainly on cardiovascular-related diseases, but with mixed results (14). An initial study in percutaneous coronary intervention patients treated with DAPT showed that the A allele carriers of rs12041331 experienced cardiovascular events (HR = 2.62; 95% CI, 0.96–7.10; $P = 0.059$) or death (HR = 3.97; 95% CI, 1.10–14.31; $P = 0.035$) more frequently compared to GG homozygotes (20). Xu et al. assessed the AA homozygotes of PEAR1 rs12041331 and its relation to clinical outcome in over 2,400 Chinese population receiving DAPT after percutaneous coronary intervention. They found that these patients had an almost equal to three-fold increase in 30-day incidence of major adverse cardiovascular events risk compared with non-AA homozygotes (15). However, these results were

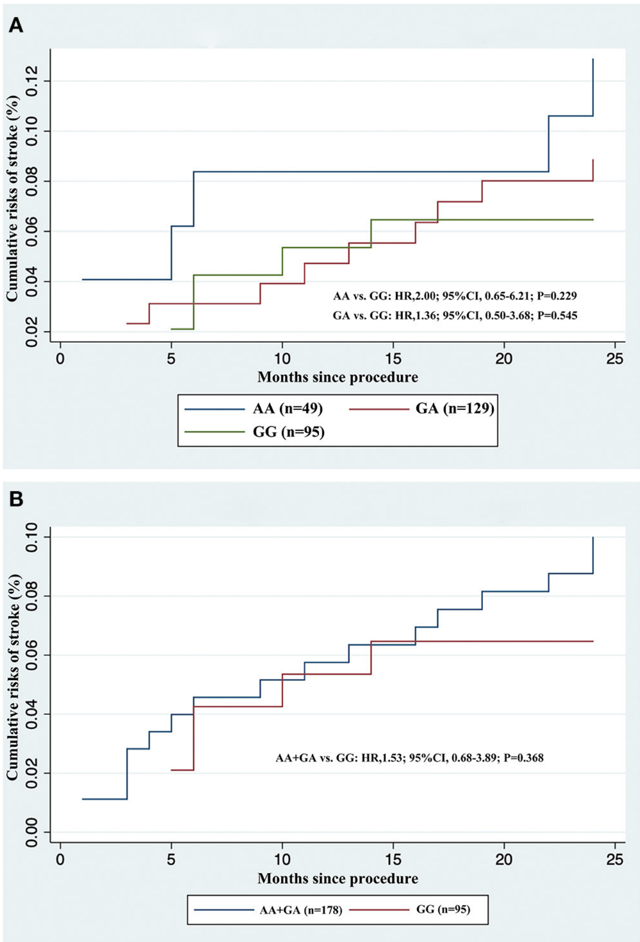

FIGURE 3 | Cumulative incidences of recurrent stroke in acute minor ischemic stroke and transient ischemic attack patients treated with dual antiplatelet therapy, stratified by rs12041331 genotypes. **(A)** AA or GA carriers vs. GG wild type. **(B)** AA + GA carriers vs. GG wild type.

not confirmed in an Egyptian acute coronary syndrome patient treated with DAPT, which reported no association with PEAR1 rs12041331 and cardiovascular risks (21). Moreover, the Aspirin in Reducing Events in the Elderly trial analyzed the relationship between PEAR1 rs12041331 and cardiovascular outcomes in a healthy elderly population with no previous atherothrombotic cardiovascular disease (14). After a median follow-up of 4.7 years, they found no significant interaction effects between the A allele carriers of rs12041331 and cardiovascular events regardless of aspirin use. This study showed that PEAR1 rs12041331 was not an important contributor to clinical events in the context of primary prevention. Consistent with its role in secondary prevention with aspirin, it does not make a contribution to clinical events in primary prevention.

At present, there are few studies on PEAR1 rs12041331 genotype in stroke. Most recently, Peng et al. explore PEAR1 rs12041331 with the platelet activity in 283 Chinese ischemic stroke patients receiving aspirin therapy, and no association was observed between platelet activity during aspirin therapy and rs12041331 (22). Zhao et al. assessed retrospectively the rs12041331 in 56 patients with recurrent ischemic stroke and 137 patients with initial stroke. They found that rs12041331 was independently associated with recurrent ischemic stroke, and the A allele showed a higher frequency than the G allele in the recurrent ischemic stroke group (17). The above-mentioned cases were the only two studies focused on the correlation between PEAR1 rs12041331 and stroke, both of which are retrospective. However, no studies have identified the impact of PEAR1 rs12041331 on the prognosis of acute stroke.

Given that aspirin and clopidogrel are the first-line treatments for the secondary prevention of atherothrombotic events in minor stroke or TIA patients and the effect of PEAR1 rs12041331 on platelet aggregation (8, 9) and in response to antiplatelet agents (10, 11), the present study has been undertaken in patients with minor stroke or TIA, which is the first research on PEAR1 rs12041331 genotype and long-term cerebrovascular outcomes conducted to date. In our investigation, we did not observe a significant association between PEAR1 rs12041331 genotype and long-term cerebrovascular events, bleeding events, and clinical function in the entire cohort. Inconsistent with those of Zhao et al. (17), our results suggested that rs12041331 genetic polymorphism is not an important contributor to clinical events in AMIS and TIA patients in the setting of secondary prevention.

Potential Mechanism

It is necessary to clarify the potential mechanisms of clinical outcome difference response to the PEAR1 rs12041331 genotype. Individual differences in drug metabolism, response, and toxicity in humans were considered to be correlated with gene polymorphism. Besides this, ethnic differences in the PEAR1 gene polymorphism are one of the most important factors, which should be considered to explain the clinical outcome differences. Moreover, cell-specific PEAR1 methylation reveals a locus that coordinates the expressions of multiple genes (23), which may provide an explanation for the diversity of clinical events. Meanwhile, methylation is greatly influenced by environmental factors, which may constantly affect the final events. In addition, although PEAR1 rs12041331 was among the strongest determinants of platelet aggregation pre-aspirin administration, it could only account for ∼15% of the total phenotypic variation in platelet function (24). In view of the fact that the occurrence of clinical events is often caused by multiple factors, exploring only one certain variable may not be enough to get a positive outcome.

Clinical Consideration

While studies have identified some genetic determinants of inter-individual variability in on-treatment platelet inhibition (e.g., PEAR1), evidence on whether these variants have clinical value to predict vascular events remains controversial. A previous study found a dose–response relation between the expression of PEAR1 protein and the number of G alleles at rs12041331 in response to several agonists in human platelets (19). However, most cardiovascular studies on PEAR1 rs12041331 genotype have found that rs12041331 A allele is more prone to cardiovascular events. As a preliminary observational study, we found no impact of PEAR1 rs12041331 on the prognosis of minor stroke and TIA. Due to the low number of patients and events, these results should also be interpreted with caution, and further analysis of other large research is therefore warranted.

Strengths and Limitations

This is a study that assessed the association of PEAR1 rs12041331 genetic polymorphism and the long-term cerebrovascular events, bleeding events, and clinical functions in patients with minor stroke or TIA. The results from this study will provide a reference for the relationship between genetic susceptibility of anti-platelet aggregation therapy and cerebrovascular risks in clinical practice. This study has some limitations that should be highlighted. First, the data that we collected were from a single center, so the sample size was not large enough, which might limit the generalizability of our findings. Second, our study is limited to the reporting of long-term clinical outcomes, lacking on-treatment platelet reactivity, which can more intuitively reflect the risks of thrombosis. Third, PEAR1 rs12041331 was reported to be more associated with platelet aggregation of aspirin, but the subjects of our study were treated with DAPT, not aspirin alone, so we cannot exclude the effects of other pivotal genes related to clopidogrel on outcomes, such as CYP2C19 polymorphisms, which are fully known to affect the platelet reactivity of clopidogrel. Future studies, after adjusting for other gene polymorphisms like CYP2C19, are needed to explore the role of PEAR1 rs12041331.

CONCLUSION

We could not replicate the previous findings suggesting that A allele carriers of PEAR1 rs12041331 were an important genetic determinant of clinical atherothrombotic or bleeding events. Our data do provide robust evidence that genetic variation in PEAR1 rs12041331 does not contribute to atherothrombotic or bleeding risk in minor stroke and TIA patients treated with DAPT.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Yangpu Hospital, Tongji University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Y-ML and Y-HY conceived and designed this study. X-GZ, Q-QF, S-WC, S-SJ, and JX were involved in the acquisition and interpretation of the data. X-GZ, J-YG, and Q-QF wrote the manuscript with contributions from all the authors. Y-MK and Y-HY refined the manuscript. All the authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.649056/full#supplementary-material

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Safety and Efficacy of Low-Dose Tirofiban Combined With Intravenous Thrombolysis and Mechanical Thrombectomy in Acute Ischemic Stroke: A Matched-Control Analysis From a Nationwide Registry

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Purpose: Tirofiban administration to acute ischemic stroke patients undergoing mechanical thrombectomy with preceding intravenous thrombolysis remains controversial. The aim of the current study was to evaluate the safety and efficacy of low-dose tirofiban during mechanical thrombectomy in patients with preceding intravenous thrombolysis.

Methods: Patients with acute ischemic stroke undergoing mechanical thrombectomy and preceding intravenous thrombolysis were derived from "ANGEL-ACT," a multicenter, prospective registry study. The patients were dichotomized into tirofiban and non-tirofiban groups based on whether tirofiban was administered. Propensity score matching was used to minimize case bias. The primary safety endpoint was symptomatic intracerebral hemorrhage (sICH), defined as an intracerebral hemorrhage (ICH) associated with clinical deterioration as determined by the Heidelberg Bleeding Classification. All ICHs and hemorrhage types were recorded. Clinical outcomes included successful recanalization, dramatic clinical improvement, functional independence, and mortality at the 3-month follow-up timepoint. Successful recanalization was defined as a modified Thrombolysis in Cerebral Ischemia score of 2b or 3. Dramatic clinical improvement at 24 h was defined as a reduction in NIH stroke score of $\geq$10 points compared with admission, or a score $\leq$1. Functional independence was defined as a Modified Rankin Scale (mRS) score of 0–2 at 3-months.

Results: The study included 201 patients, 81 in the tirofiban group and 120 in the non-tirofiban group, and each group included 68 patients after propensity score matching. Of the 201 patients, 52 (25.9%) suffered ICH, 15 (7.5%) suffered sICH, and 18 (9.0%) died within 3-months. The median mRS was 3 (0–4), 99 (49.3%) achieved functional independence. There were no statistically significant differences in safety outcomes, efficacy outcomes on successful recanalization, dramatic clinical improvement, or 3-month mRS between the tirofiban and non-tirofiban groups (all $p > 0.05$). Similar results were obtained after propensity score matching.

Conclusion: In acute ischemic stroke patients who underwent mechanical thrombectomy and preceding intravenous thrombolysis, low-dose tirofiban was not associated with increased risk of sICH or ICH. Further randomized clinical trials are needed to confirm the effects of tirofiban in patients undergoing bridging therapy.

Keywords: tirofiban, mechanical thrombectomy, intravenous thrombolysis, large vessel occlusion, propensity score matching

INTRODUCTION

Endovascular treatment has proved to be effective for improving functional outcomes and reducing mortality in patients with large-artery occlusive stroke (1–7). However, during the operative procedure, platelet aggregation caused by severe atherosclerotic stenosis or endothelial damage can lead to thrombotic events and early re-occlusion (8, 9). The highly selective glycoprotein IIb/IIIa receptor antagonist tirofiban can efficiently block the final pathway of platelet aggregation and subsequent thrombus formation (10).

A number of studies have reported the effects of tirofiban during mechanical thrombectomy (MT), but outcomes are controversial (11–14). One of the main concerns is whether tirofiban will lead to increased risks of bleeding in patients who have received intravenous thrombolysis (IVT) before MT. Because of this, the use of antiplatelet agents is not recommended within 24 h after IVT in the American Heart Association/American Stroke Association (AHA/ASA) guidelines (15). Few prospective studies have focused on tirofiban administration during MT in patients with preceding IVT, which is also known as bridging therapy. The aim of the current prospective multicenter study was to evaluate the safety of tirofiban during MT with respect to symptomatic intracerebral hemorrhage (sICH) and intracerebral hemorrhage (ICH), as well as its efficacy during artery recanalization, and functional outcomes in patients who underwent bridging IVT.

MATERIALS AND METHODS

Patient Enrolment

All patients were enrolled from the registry of "ANGEL-ACT," which was a nationwide, multicenter, prospective registry study conducted in China from November 2017 to March 2019. Details of the design of the ANGEL-ACT have been reported previously (16). The protocol of the ANGEL-ACT was approved by the Ethics Committee of Beijing Tiantan Hospital and all other participating centers. Written informed consent was obtained from all patients or their representatives. The current study included the following data: (1) Anterior circulation large vessel occlusion (ICA/M1); (2) onset to groin time ≤6 h; (3) the Alberta Stroke Program Early CT Score (ASPECTS) ≥6; and (4) underwent thrombolytic therapy. The main exclusion criterion was incomplete clinical data.

Endovascular Interventions and Grouping

In all MTs a stent retriever or aspiration device was the first recanalization option, in accordance with protocol. In cases in which the first recanalization failed, additional thrombectomy attempts and alternative rescue therapies were used at the discretion of the operator, including intra-arterial or intravenous tirofiban administration, intra-arterial thrombolysis, balloon angioplasty, and emergent stenting. The patients were divided into a tirofiban group and a non-tirofiban group based on tirofiban administration during MT.

Tirofiban Administration During Mechanical Thrombectomy

All eligible patients underwent endovascular treatment immediately after the assessment of indications. In general, tirofiban was given under the following conditions: (1) Emergency stenting for severe residual stenosis or instant re-occlusion; (2) balloon angioplasty for severe residual stenosis or instant re-occlusion; (3) successful mechanical recanalization with three or more passes with a stent retriever for presumed endothelial damage or instant re-occlusion; and (4) severe *in situ* atherosclerosis with a high risk of early re-occlusion. Unless an ICH was suspected, a low-dose intra-arterial bolus (0.25–1.00 mg) followed by a continuous intravenous infusion (0.1 μg/kg/min) was administrated for 24 h as a standard procedure. At 4 h prior to the end of the infusion, dual antiplatelet agents (aspirin 100 mg and clopidogrel 75 mg) were administered as bridging therapy if ICH was excluded within 24 h via follow-up computed tomography or magnetic resonance imaging.

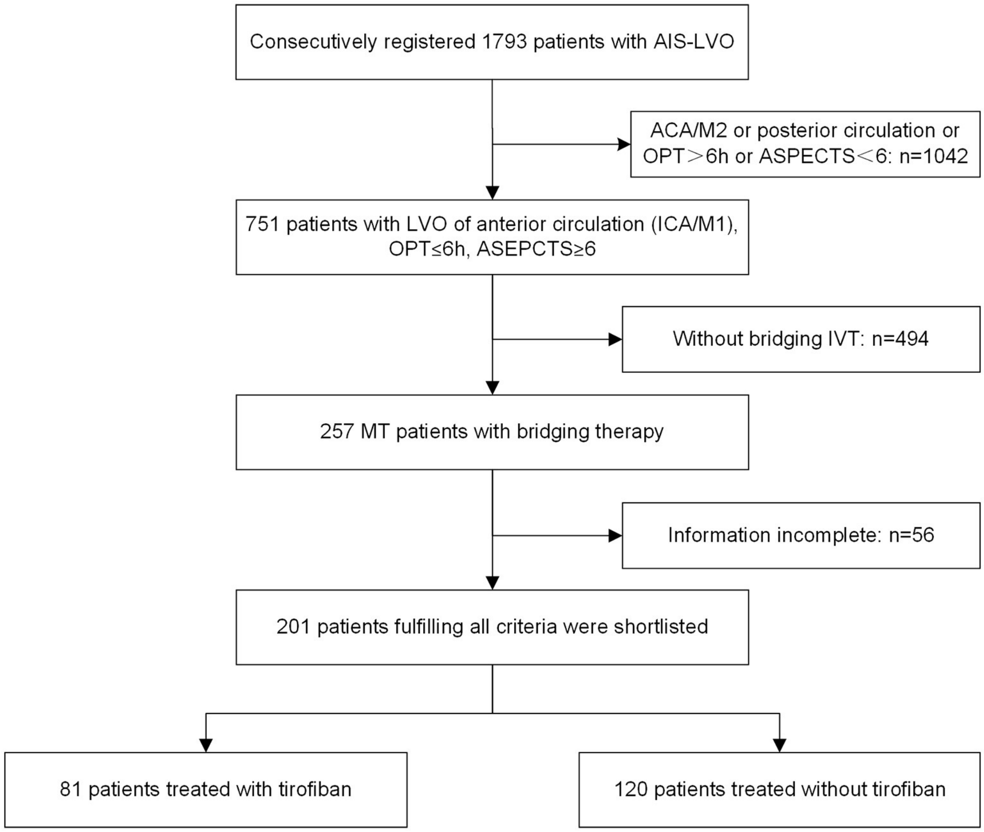

FIGURE 1 | Flowchart. ACA, anterior cerebral artery; AIS, acute ischemic stroke; ASPECTS, Alberta Stroke Program Early CT Score; ICA, internal carotid artery; IVT, intravenous thrombolysis; LVO, large vessel occlusion; M1, M1 segment; M2, M2 segment; MT, mechanical thrombectomy; and OPT, onset-to-puncture time.

Safety and Efficacy Outcomes

The main safety endpoints were sICH, ICH, and mortality within 3-months. sICH was defined as an ICH associated with clinical deterioration according to the Heidelberg Bleeding Classification (17). Hemorrhage types were also recorded. Hemorrhagic outcomes were assessed by a core laboratory, blinded to the clinical data and outcomes. Efficacy outcomes included successful recanalization, dramatic clinical improvement, and functional independence. Successful recanalization was defined as a modified Thrombolysis in Cerebral Ischemia (mTICI) score of 2b or 3 (18). Dramatic clinical improvement was defined at 24 h as a reduction in NIH Stroke Scale (NIHSS) score of $\geq$ 10 points compared with admission, or a score of $\leq$1 (19). Functional independence was defined as a modified Rankin Scale (mRS) score of 0–2 at 3-months.

Statistical Analysis

Baseline patient demographic information in the tirofiban and non-tirofiban groups were compared, as were all endpoints. A logistic regression model was used to investigate associations between tirofiban administration and safety and efficacy endpoints. To reduce data bias and confounding variables, propensity score matching (PSM) analysis was performed by matching patients in the two groups at a 1:1 ratio. Age, sex, baseline Modified Rankin Scale score, baseline NIHSS score, ASPECTS score, onset-to-puncture time, and pathogenesis of stroke were used to generate a propensity score for each subject. After PSM the two groups were again compared via the aforementioned statistical methods.

For continuous data, means $\pm$ standard deviation or medians and interquartile ranges were used to summarize data, and two-sided t-tests for independent samples or Mann-Whitney U tests were used to assess the significance of differences between groups. Frequencies and percentages were used to summarize binary data, and between-group comparisons were performed via the $\chi 2$ test or Fisher's exact tests as appropriate. All analyses were performed using SAS version 9.4 software (SAS Institute, Cary, NC, USA), and $p < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

A total of 1,793 consecutive patients who underwent endovascular treatment were initially recruited from the ANGEL-ACT registry, of which 201 were subsequently shortlisted based on the above-described criteria (**Figure 1**); 81 in the tirofiban group and 120 in the non-tirofiban group. The median age of the 201 patients was 64 years (range 55–70

TABLE 1 | Baseline characteristics of patients before PSM.

Variable	All patients (n = 201)	Tirofiban (n = 81)	Non-tirofiban (n = 120)	*P*-value
Age, median (IQR)	64 (55–70)	62 (53–69)	65 (57–70)	0.104
Male sex, *n* (%)	130 (64.7)	52 (64.2)	78 (65.0)	1.000
Initial NIHSS score, median (IQR)	14 (11–18)	15 (13–19)	14 (11–18)	0.416
Medical history				
Atrial fibrillation, *n* (%)	70 (34.8)	22 (27.2)	48 (40.0)	0.071
Hypertension, *n* (%)	93 (46.3)	45 (55.6)	48 (40.0)	0.032
Diabetes mellitus, *n* (%)	29 (14.4)	16 (19.8)	13 (10.8)	0.101
Hypercholesterolemia, *n* (%)	16 (8.0)	8 (9.9)	8 (6.7)	0.435
Ischemic stroke, *n* (%)	28 (13.9)	9 (11.1)	19 (15.8)	0.409
Smoking, *n* (%)	77 (38.3)	32 (39.5)	45 (37.5)	0.883
Prior antiplatelet use, *n* (%)	23 (11.4)	9 (11.1)	14 (11.7)	1.000
Prior anticoagulant use, *n* (%)	1 (0.5)	0 (0.0)	1 (0.8)	1.000
Pre-stroke mRS score, *n* (%)				0.086
0	189 (94.0)	79 (97.5)	110 (91.7)	
1	12 (6.0)	2 (2.5)	10 (8.3)	
Stroke causative mechanism, *n* (%)				0.011
Large artery atherosclerosis	85 (42.3)	44 (54.3)	41 (34.2)	
Cardioembolism	87 (43.3)	28 (34.6)	59 (49.2)	
Other	29 (14.4)	9 (11.1)	20 (16.6)	
ASPECTS, median (IQR)	10 (8–10)	10 (8–10)	10 (8–10)	0.611
Treatment profiles				
General anesthesia, *n* (%)	59 (29.4)	24 (29.6)	35 (29.2)	1.000
Number of pass, median (IQR)	2.6±1.7	2.7±1.5	2.5±1.8	0.110
Heparin during MT, *n* (%)	85 (42.3)	28 (34.6)	57 (47.5)	0.081
IA thrombolysis, *n* (%)	7 (3.5)	3 (3.7)	4 (3.3)	1.000
Permanent stenting, *n* (%)	34 (16.9)	19 (23.5)	15 (12.5)	0.055
Transfer from primary stroke center, *n* (%)	68 (33.8)	44 (36.7)	24 (29.6)	0.362
OPT time, median (IQR), min	245 (200–294)	255 (218–302)	241 (194–289)	0.056
PRT time, median (IQR), min	80 (52–125)	78 (52–128)	80 (52–119)	0.908

ASPECTS, Alberta Stroke Program Early CT Score; IA, intraarterial; IQR, interquartile range; mRS, modified Rankin Scale; MT, mechanical thrombectomy; NIHSS, National Institutes of Health Stroke Scale; OPT, onset-to-puncture time; and PRT, puncture-to-recanalization time.

years), and 130 (64.7%) were male. Patients in the tirofiban group exhibited a significantly heavier atherosclerotic burden with respect to vascular risk factors such as hypertension (55.6% vs. 40.0%, $p = 0.032$), and were more likely to have a large-artery atherosclerotic stroke (54.3% vs. 34.2%) (**Table 1**). Sixty-eight patients from each group were included in the PSM analysis. The comparison of baseline characteristics between the two groups after PSM is shown in **Table 2**. Both groups were comparable with respect to baseline characteristics. Initial NIHSS score, IV thrombolysis, medical history, and mechanism of stroke were similar in both groups.

Safety Outcomes

Fifteen (7.5%) patients suffered sICH within 24 h after MT, and 52 (25.9%) experienced ICH. There were no significant between-group differences in the incidences of sICH, any ICH, or mortality within 3-months in the entire cohort (all $p > 0.05$). In the PSM cohort the findings were similar. Three (4.4%) patients in the tirofiban group and seven (10.3%) in the non-tirofiban group suffered sICH ($p > 0.05$). Fifteen (22.1%) patients in the tirofiban group and 25 (36.8%) in the non-tirofiban group experienced any ICH ($p > 0.05$). A total of 15 (11.0%) patients died after 3-months, 5 (7.4%) in the tirofiban group and 10 (14.7%) in the non-tirofiban group ($p > 0.05$) (**Tables 3**, **4**).

Efficacy Outcome

Overall, 185 (92.0%) patients who underwent IVT bridging therapy experienced successful recanalization, 74 (91.4%) in the tirofiban group and 111 (92.5%) in the non-tirofiban group (adjusted $p = 0.652$). The successful recanalization rates in the tirofiban group and the non-tirofiban group did not differ significantly after PSM (adjusted $p = 0.993$). In the entire cohort the median NIHSS score at 24 h post-MT was 9 (range 3–14). Sixty-five (32.3%) patients exhibited marked clinical improvement, 27 (33.3%) in the tirofiban group and 38 (31.7%) in the non-tirofiban group. At the 3-month follow-up timepoint, 99 (49.3%) patients had reached functional independence, 40

TABLE 2 | Baseline characteristics of patients after PSM.

Variable	Tirofiban (n = 68)	Non-tirofiban (n = 68)	*P*-value
Age, median (IQR)	62 (54–70)	62 (53–69)	0.984
Male sex, *n* (%)	43 (63.2)	44 (64.7)	1.000
Initial NIHSS score, median (IQR)	15 (12–19)	15 (10–19)	0.877
Medical history			
Atrial fibrillation, *n* (%)	22 (32.4)	26 (38.2)	0.591
Hypertension, *n* (%)	36 (52.9)	25 (36.8)	0.084
Diabetes mellitus, *n* (%)	13 (19.1)	9 (13.2)	0.486
Hypercholesterolemia, *n* (%)	5 (7.4)	5 (7.4)	1.000
Ischemic stroke, *n* (%)	9 (13.2)	12 (17.7)	0.636
Smoking, *n* (%)	26 (38.2)	28 (41.2)	0.861
Prior antiplatelet use, *n* (%)	7 (10.3)	8 (11.8)	1.000
Prior anticoagulant use, *n* (%)	0 (0.0)	1 (1.5)	1.000
Pre-stroke mRS score, *n* (%)			1.000
0	66 (97.1)	65 (95.6)	
1	2 (2.9)	3 (4.4)	
Stroke causative mechanism, *n* (%)			0.608
Large artery atherosclerosis	32 (47.1)	30 (44.1)	
Cardioembolism	27 (39.7)	32 (47.1)	
Other	9 (13.2)	6 (8.8)	
ASPECTS, median (IQR)	10 (8–10)	10 (8–10)	0.802
Treatment profiles			
General anesthesia, *n* (%)	19 (27.9)	22 (32.3)	0.709
Number of pass, median (IQR)	3 (2–4)	2 (1–3)	0.169
Heparin during MT, *n* (%)	24 (35.3)	27 (39.7)	0.723
IA thrombolysis, *n* (%)	3 (4.4)	2 (2.9)	1.000
Permanent stenting, *n* (%)	14 (20.6)	9 (13.2)	0.361
Transfer from primary stroke center, *n* (%)	21 (30.9)	26 (38.2)	0.471
OPT time, median (IQR), min	253 (208–301)	255 (215–293)	0.969
PRT time, median (IQR), min	80 (53–130)	81 (52–117)	0.686

ASPECTS, Alberta Stroke Program Early CT Score; IA, intraarterial; IQR, interquartile range; mRS, modified Rankin Scale; MT, mechanical thrombectomy; NIHSS, National Institutes of Health Stroke Scale; OPT, onset-to-puncture time; and PRT, puncture-to-recanalization time.

(49.4%) in the tirofiban group and 59 (49.2%) in the non-tirofiban group (**Figure 2**). There were no significant differences in any of the above outcomes between the two groups (all $p > 0.05$). Consistent results were observed in the PSM analysis.

DISCUSSION

In the current prospective registry study, low-dose tirofiban during MT with bridging IVT exhibited acceptable safety with respect to sICH and ICH. The ICH rate was lower in the tirofiban group, but not significantly before or after PSM. This suggested that low-dose tirofiban may be a safe alternative therapy during MT in patients with bridging IVT, especially those with severe *in situ* atherosclerotic stenosis, permanent stenting, or obvious endothelial damage.

Tirofiban is a non-peptide antagonist of the glycoprotein IIb/IIIa receptor, which regulates the final pathway of platelet aggregation (10). To date little high-quality research has focused on the effects of therapy with glycoprotein IIb/IIIa receptor antagonists during MT in patients with bridging IVT. Huo et al. (20) reported the safety of tirofiban in patients who underwent bridging therapy, but did not detect benefits on long-term functional outcomes. In contrast, Kellert et al. (13) concluded that tirofiban was associated with a higher risk of fatal ICH and poorer outcomes, regardless of whether preceding IVT was administered or not. Notably however, the two studies were observational studies with uncontrolled experimental designs, limited sample sizes, and heterogeneous treatment modalities, thus caution is advised when generalizing from their results.

The use of tirofiban was at the discretion of the treating physician and local practice in the present study. Consistent with previous studies, large-artery atherosclerotic stroke pathogenesis was significantly higher in the tirofiban group ($p = 0.011$) before PSM. It may be more difficult to achieve successful recanalization in patients with underlying atherosclerotic stenosis, and re-occlusion is more common, so tirofiban with or without

TABLE 3 | Safety and efficacy endpoints of MT patients with preceding intravenous thrombolysis before PSM.

	All patients	Tirofiban	Non-tirofiban	*P*-value	OR	Adjusted *P*-value*	Adjusted OR*
sICH	15 (7.5)	5 (6.2)	10 (8.3)	0.785	0.72 (0.24, 2.20)	0.682	0.77 (0.21, 2.75)
Any ICH	18 (22.2)	34 (28.3)	52 (25.9)	0.412	0.72 (0.38, 1.40)	0.526	0.78 (0.36, 1.68)
Hemorrhage type, *n* (%)				0.732	NA	NA	NA
HI	33 (63.5)	13 (72.2)	20 (58.8)				
PH1	8 (15.4)	1 (5.6)	7 (20.6)				
PH2	9 (17.3)	2 (11.1)	7 (20.6)				
rPH	1 (1.9)	1 (5.6)	0 (0.0)				
IVH	0 (0.0)	0 (0.0)	0 (0.0)				
SAH	1 (1.9)	1 (5.6)	0 (0.0)				
Successful recanalization	185 (92.0)	74 (91.4)	111 (92.5)	0.769	0.86 (0.31, 2.40)	0.652	0.76 (0.23, 2.50)
Dramatic clinical improvement	65 (32.3)	27 (33.3)	38 (31.7)	0.878	1.08 (0.59, 1.97)	0.344	1.43 (0.68, 2.98)
3-month mRS, median (IQR)	3 (0–4)	3 (0–4)	3 (0–4)	0.595	1.15 (0.70, 1.89)	0.474	1.23 (0.70, 2.15)
3-month mRS 0–2	99 (49.3)	40 (49.4)	59 (49.2)	1.000	1.01 (0.57, 1.77)	0.921	1.03 (0.54, 1.97)
3-month mortality	18 (9.0)	6 (7.4)	12 (10.0)	0.620	0.72 (0.26, 2.00)	0.603	0.73 (0.23, 2.36)

ASPECTS, Alberta Stroke Program Early CT Score; HI, hemorrhagic infarction; ICH, intracranial hemorrhage; IQR, interquartile range; IVH, intraventricular hemorrhage; mRS, modified Rankin Scale; MT, mechanical thrombectomy; NA, not applicable; NIHSS, National Institutes of Health Stroke Scale; OPT, onset-to-puncture time; OR, odds ratio; PH, parenchymal hemorrhage; rPH, remote from infarcted brain tissue; SAH, subarachnoid hemorrhage; and sICH, symptomatic intracranial hemorrhage.

TABLE 4 | Safety and efficacy endpoints of MT patients with preceding intravenous thrombolysis after PSM.

	Tirofiban	Non-tirofiban	*P*-value	OR	Adjusted *P*-value*	Adjusted OR*
sICH	3 (4.4)	7 (10.3)	0.325	0.40 (0.10–1.63)	0.362	0.50 (0.11–2.25)
Any ICH	15 (22.1)	25 (36.8)	0.090	0.49 (0.23–1.04)	0.100	0.47 (0.19–1.16)
Hemorrhage type, *n* (%)			0.679	NA	NA	NA
HI	12 (80.0)	16 (64.0)				
PH1	0 (0.0)	5 (20.0)				
PH2	1 (6.7)	4 (16.0)				
rPH	1 (6.7)	0 (0.0)				
IVH	0 (0.0)	0 (0.0)				
SAH	1 (6.7)	0 (0.0)				
Successful recanalization	61 (89.7)	63 (92.7)	0.547	0.69 (0.21–2.30)	0.993	1.01 (0.22–4.68)
Dramatic clinical improvement	22 (32.4)	18 (26.5)	0.573	1.33 (0.63–2.79)	0.552	1.30 (0.54–3.13)
3-month mRS, median (IQR)	3 (0–4)	3 (0–5)	0.264	1.41 (0.78–2.56)	0.545	1.23 (0.64–2.36)
3-month mRS 0–2	34 (50.0)	31 (45.6)	0.732	1.19 (0.61–2.34)	0.744	1.14 (0.52–2.50)
3-month mortality	5 (7.4)	10 (14.7)	0.273	0.46 (0.15–1.43)	0.862	0.88 (0.22–3.55)

ASPECTS, Alberta Stroke Program Early CT Score; HI, hemorrhagic infarction; ICH, intracranial hemorrhage; IQR, interquartile range; IVH, intraventricular hemorrhage; mRS, modified Rankin Scale; MT, mechanical thrombectomy; NA, not applicable; NIHSS, National Institutes of Health Stroke Scale; OPT, onset-to-puncture time; OR, odds ratio; PH, parenchymal hemorrhage; rPH, remote from infarcted brain tissue; SAH, subarachnoid hemorrhage; and sICH, symptomatic intracranial hemorrhage.

**Adjusted for age, baseline mRS score, baseline NIHSS score, ASPECTS, atrial fibrillation, hypertension, pathogenesis of stroke, heparin during MT, permanent stenting, OPT.*

angioplasty as an adjuvant rescue strategy may be required. This is concordant with the higher incidence of stent placement in the tirofiban group (23.5% vs. 12.5%, $p = 0.055$).

In combination therapy with intravenous thrombolysis, Zinkstok et al. (21) suggested that early intravenous administration of aspirin shortly after rt-PA was significantly associated with a higher risk of sICH in the Antiplatelet Therapy in Combination With rt-PA Thrombolysis in Ischemic Stroke trial. Based on this, the use of antiplatelet agents is not recommended within 24 h after IVT in the AHA/ASA guidelines because of the concern of increased hemorrhagic complications (15). Notably however, different inhibition modalities and biologic half-lives influence responses to medication-induced bleeding. Tirofiban is a highly selective and reversible glycoprotein IIb/IIIa receptor antagonist, and has been proven to be safe within the first 24 h after IVT (22). Low-dose tirofiban has been selectively used as rescue therapy during MT in patients with endothelial damage or *in situ* atherosclerotic stenosis in our clinical practice, and has exhibited acceptable safety. The current study preliminarily confirmed the safety of low-dose tirofiban during MT with respect to sICH and ICH in patients with preceding IVT.

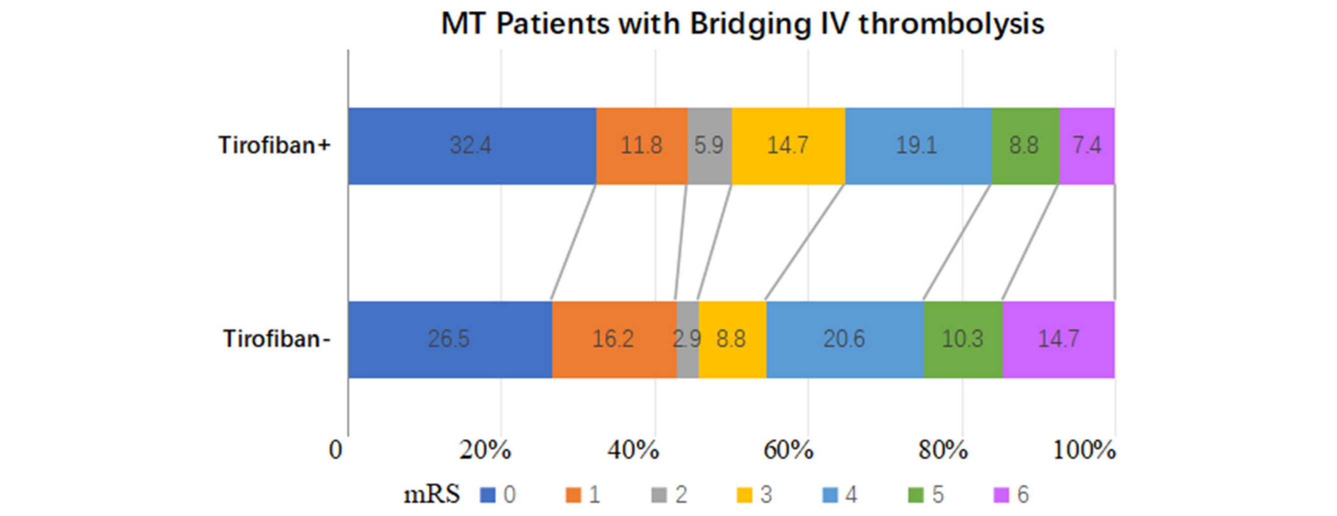

FIGURE 2 | Distributions of the 3-month mRS of patients who underwent mechanical thrombectomy and preceding intravenous thrombolysis after PSM.

The results of the current study differ from those reported by Kellert et al. (13) and Wu et al. (23) with regard to the safety of rescue tirofiban during MT. This might be due to the following reasons. One pertains to the dosage of tirofiban administration during MT. We reviewed all studies on tirofiban dosage during endovascular treatment of LVO (24). Based on this, we introduced a low-dose intra-arterial bolus of tirofiban (0.25–1.00 mg) for rapid effects on angiographic changes, followed by a continuous intravenous infusion at the lower rate of 0.1 μg/kg/min for 24 h as a standard procedure. Second, according to the specific inhibitory effect on platelet aggregation and atherothrombosis of tirofiban, we prespecified the indications for tirofiban administration during MT in the protocol. Thus, tirofiban was more selectively utilized for large-artery atherosclerotic infarction rather than cardio-embolic stroke (54.3% vs. 34.6%), which might reduce the risk of bleeding. Notably, some of the clinical characteristics of the tirofiban group differed from those of the non-tirofiban group before PSM, which may have affected outcomes. Consequently, PSM was applied to reduce the influence of confounding variables.

The current study had several limitations. First and foremost, all subjects were from an observational study. PSM analysis and a multivariable logistic regression model were used in an effort to reduce selection bias, but potential confounders cannot be ruled out despite adjustment and matching. Therefore, the results of the study need to be interpreted carefully, particularly given that the rate of sICH was lower in the tirofiban group after PSM. Another potential limitation was that all subjects were from China, which has a high prevalence of intracranial atherosclerosis (25). Thus, the results of the study may not be directly generalizable to other populations.

CONCLUSION

In summary, low-dose tirofiban during MT was not associated with an increased risk of sICH or ICH in patients with preceding IVT. Further dose-escalation trials are needed to confirm its safety and efficacy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IRB of Beijing Tiantan Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ZM designed, led the study, had full access to all of the data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis. GM and SL prepared the first draft of the report. AW and YSP did statistical analyses. All authors except AW and YSP participated in patient enrolment and collection of data. All authors critically reviewed the report and approved the final version.

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REVIEW
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Biomarkers for Antiplatelet Therapies in Acute Ischemic Stroke: A Clinical Review

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Stroke is one of the world's leading causes of disability and death. Antiplatelet agents are administered to acute ischemic stroke patients as secondary prevention. Clopidogrel involves biotransformation by cytochrome P450 (CYP) enzymes into an active metabolite, and single nucleotide polymorphisms (SNPs) can influence the efficacy of this biotransformation. Despite the therapeutic advantages of aspirin, there is significant inter-individual heterogeneity in response to this antiplatelet drug. In this clinical review, the recent advances in the biomarkers of antiplatelet agents in acute ischemic stroke are discussed. The studies reviewed herein highlight the clinical relevance of antiplatelet resistance, pharmacotherapy of antiplatelet agents predicting drug response, strategies for identifying aspirin resistance, pharmacogenetic variants of antiplatelet agents, miRNAs, and extracellular vesicles (EVs) as biomarkers toward the personalized approach in the management of acute ischemic stroke. The precise pathways contributing to antiplatelet resistance are not very well known but are presumably multi-factorial. It is essential to understand the clinical relevance of clopidogrel and aspirin-related single nucleotide polymorphism (SNPs) as potential predictive and prognostic biomarkers. Prasugrel is a next-generation antiplatelet agent that prevents ADP-platelet activation by binding irreversibly to P2Y12 receptor. There are sporadic reports of prasugrel resistance and polymorphisms in the Platelet endothelial aggregation receptor-1 (PEAR1) that may contribute to a change in the pharmacodynamics response. Ticagrelor, a direct-acting P2Y12-receptor antagonist, is easily absorbed and partly metabolized to major AR-C124910XX metabolite (ARC). Ticagrelor's primary active metabolite, ARC124910XX (ARC), is formed via the most abundant hepatic cytochrome P450 (CYP) enzyme, CYP3A4, and CYP3A5. The integration of specific biomarkers, genotype as well as phenotype-related data in antiplatelet therapy stratification in patients with acute ischemic stroke will be of great clinical significance and could be used as a guiding tool for more effective, personalized therapy.

Keywords: aspirin, clopidgrel, stroke, prasugrel, ticagrelor, biomarkers, ischemic stroke, resistance

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INTRODUCTION

Acute ischemic stroke (AIS) is an atherosclerotic arterial disease, which is the major cause of death worldwide, leading to an estimated 5.5 million deaths each year (1). The etiology of stroke is established to be multi-factorial. Antiplatelet therapy plays a major role in the primary and secondary prevention of AIS. Most of the stroke occurrence is ischemic and is commonly due to the formation and traveling of the formed emulous into the large vessels, which compromises the blood flow into the brain (2). Neuroimaging is the technique used in the diagnosis and management of the AIS. It plays a major role, as it helps in the differentiation of the hemorrhagic and ischemic stroke where it is important in further management (3). Despite the therapeutic advances in recurrent ischemic stroke management, it affects the quality of life in most people. The treatment failure occurs due to resistance toward antiplatelet therapy or clinically referred to as high on-treatment platelet reactivity (HTPR) (4–6). To overcome this, many platelet function tests are being used, which helps in the platelet function guided antiplatelet therapy, i.e., personalized antiplatelet therapy (7, 8). In recent years, the use of novel biomarkers and pharmacogenetic related data correlating the antiplatelet response and translating it to clinical care has been an area of focus. The incorporation of genomics data along with the clinical markers will be of a paradigm shift in personalized neurology. Hence, this review focuses on interindividual variability and discusses the significance of novel biomarkers and pharmacogenetic data toward the personalized approach in the management of acute ischemic stroke.

ACUTE ISCHEMIC STROKE (AIS)

AIS is defined as the occlusion of the brain, retina, or spinal cord supplying arteries, and this results in focal tissue infarction and corresponding sudden neurological deficits. AIS is the leading cause of death worldwide and the third major cause of disability in stroke. More than 7,00,000 cases are estimated to occur worldwide every year (1–3).

For effective diagnosis of AIS, it is important to know about the presence of etiology and risk factors. Most of the patients with etiology have more than two risk factors, and these can be modifiable or non-modifiable. The greater part of the stroke is due to embolisms from heart- cervical arteries or to the atherosclerotic plaque in the aortic arch. The most important mechanism of stroke occurs through intracranial atherosclerosis (2, 9). Based on this mechanism the etiology is subdivided into five major subtypes of (1) large-artery atherosclerosis (embolus or thromboembolism in cervical carotid arteries), (2) cardio embolism (secondary to clot formation in the heart), (3) small-vessel occlusion (lacunar infarct), (4) unusual cause or stroke of other determined causes, and (5) stroke of undetermined causes this classification is based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST), which was developed to categorize the causes of AIS (2, 9). Age is the major factor to which it varies the causes of the presence of stroke in the patients. In children, the occurrence of stroke can be following inflammatory arteriopathy infection. The age of incidence is around 39–49 years and it is higher in men than in women according to the estimate (10). Factors include the following: the presence of hypertension, an increased apolipoprotein B (Apo B) to Apo-A1 ratio, diet, psychological stress, smoking, high alcohol consumption, diabetes, chronic kidney disease, and cardiac conditions like atrial fibrillation (2, 9–13).

TABLE 1 | Etiology and parameters in diagnosis of AIS.

Etiology	Diagnostic parameter
Cardiac embolism	Echocardiography
	Holter/loop recorder
Atherosclerosis	CT angiography
	MR angiography
	Carotid Doppler ultrasonography
Small vessel disease	Brain MRI
Arterial dissection	CT angiography
	MR angiography
Cerebral vasculitis	CT angiography
	Magnetic resonance angiography
	Catheter angiography
	Cerebrospinal fluid examination
	Brain and leptomeningeal biopsy

The most important thing to note during the diagnosis is the negative factors that mimic the presence of stroke-like migraine, seizures, vestibular disturbance, metabolic disturbance, and also intracranial hemorrhage. Detection based on these symptoms is the first line for the detection of AIS (14). Globally, it is meant that computerized tomography (CT) and rapid access through magnetic resonance imaging (MRI) are the major diagnosing method used for AIS. In **Table 1**, the diagnostic parameters based on stroke etiology are mentioned (15, 16).

Pharmacotherapy of Antiplatelet Agents Predicting Drug Response

Platelet reactivity phenomena involve platelet adhesion, aggregation, and activation. Various antiplatelet agents like aspirin, clopidogrel, glycoprotein IIb/IIIa antagonists, and P2Y12 agents have been studied to prevent any events of atherothrombosis. However, variability in platelet reactivity and response between subjects is of major concern in antiplatelet therapy. It can result from a variety of factors. Elevated levels of immature platelet count and reactivity affect the response to antiplatelet agents. Drug-based factors include drug–drug interactions (DDIs), dosing, etc. Patient-related factors include compliance, metabolism, comorbidities like diabetes mellitus, obesity, abnormal lipid profile, and smoking habits. The Euro Heart Survey on Diabetes and the Heart (17) revealed patients with coronary artery disease and diabetes possess a higher risk of cardiovascular events and mortality, which explains the altered response to antiplatelet therapy (18); the concurrent occurrence of both diabetes mellitus and chronic kidney disease (CKD) increases the risk even more, creating a demand for highly effective antiplatelet treatment (18, 19). The Platelet Inhibition and Patient Outcomes (PLATO) trial comparing clopidogrel vs. ticagrelor in acute coronary syndrome (ACS) patients has revealed the possibility of harm from H2 receptor blockers with clopidogrel therapy (20). Further comparison studies have supported the use of H2 receptor blockers in the place of Proton Pump Inhibitors (PPIs) to provide GI protection, as the latter is associated with adverse health outcomes (21, 22). Moreover, recurrent strokes are instigated by homocysteine levels, where patients with higher levels show lower response to antiplatelet therapy (23–25) supported by several studies demonstrating the link between hyperhomocysteinemia and platelet activation and insufficient platelet inhibition (26). The recurrent stroke and cardiovascular events can be predicted by baseline homocysteine levels of dual antiplatelet therapy or aspirin alone in the female patients with acute minor stroke or high-risk Transient ischemic attack (TIA) (27). The CHANCE trial (Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events) demonstrated the superior benefits of dual therapy with clopidogrel and aspirin in managing recurrent stroke in patients with high-risk TIA than aspirin alone (28). Thus, in order to prevent atherothrombotic events in patients with high risk, varied antiplatelet mechanisms offered by dual antiplatelet therapy will be of huge benefit (29).

Aspirin

Several factors alter platelet reactivity and turnover and thus leading to aspirin response variability and "High on-treatment platelet reactivity" (HTPR). Hyperresponsiveness to aspirin is multifactorial with altered pathways. Ageing, type 2 diabetes mellitus (DM), and drug interactions [most common with non-steroidal anti-inflammatory agents (NSAIDs)] at binding site Ser529 of COX-1 reduce the response to aspirin (30) and proton pump inhibitors (PPIs), and myeloproliferative conditions are some of the contributing factors for variability in aspirin responses (31, 32). A variety of platelet-activating mechanisms, elevated levels of platelet production, insufficient COX-1 inhibition, augmented recovery of COX-2 with increased platelet turnover, and elevated levels of aspirin-insensitive agonists may affect the aspirin response at the cellular level. Along with these factors, genetic polymorphisms also play a vital role in altered response to aspirin between patients (33). Reduced response to aspirin is expected after coronary artery bypass graft (CABG) procedure over a brief time affecting the prevention of failure of the thrombotic graft. In such cases, aspirin dosing multiple times per day was found to control the TXB2 generation efficiently in an early study trial (34), which was confirmed by a meta-analysis including 7 Randomised Clinical Trials (RCTs), where therapy with aspirin twice daily has better antiplatelet efficacy in comparison with a daily dose of one per day (35).

Clopidogrel

This is a prodrug rendering its pharmacological action once metabolized to its active form by Cytochrome 450 and Paraoxonase-1 (PON-1). It is a two-step mechanism. The first step involves the action of CYP2C19, CYP1A2, and CYP2B6 (36). The second step involves CYP3A4, CYP2C9, and the Paraoxonase (PON-1) enzyme. Despite this, dual antiplatelet therapy is efficient in Major Adverse Cardiovascular Events (MACE) prevention and is considered as the norm in clinical management. There occurs substantial levels of recurrent events (∼10%) (37). In secondary prevention of cardio and cerebrovascular events, clopidogrel is considered a highly effective antiplatelet therapy, where along with aspirin it acts as the backbone to preventing major adverse cardiovascular events (MACE) (38). However, 25% of patients exhibit only a sub-optimal response to this drug (39). The pharmacodynamics response to clopidogrel exhibit a wide inter-individual variability (40). High platelet reactivity with clopidogrel in patients with DM leads to the impaired antiplatelet response, which is explained by the altered drug pharmacokinetics (41). CYP2C19*2 or *3 and PON-1 polymorphisms considerably diminished the platelet response to clopidogrel while the former elevates the risk of MACE in Coronary Heart Disease (CHD) patients after PCI (42). In a meta-analysis conducted with 28 studies across 17 countries in Asia, ABCB1 C3435T polymorphism considerably reduced platelet activity in patients receiving clopidogrel, thereby elevating the risk of bleeding events (43). A recent systematic review and meta-analysis study has recommended genotype testing of ABCB1 C3435T SNP for ACS/CAD patients undertaking PCI to optimize clopidogrel treatment (44). A meta-analysis study has demonstrated the risk of high PR and MACE in patients with vascular risk factors receiving clopidogrel therapy. This substantiates the need for a future individualized method of antiplatelet treatment based on the personal vascular risk factors (45).

Ticagrelor and Prasugrel

The Platelet Inhibition and Patient Outcomes (PLATO) trial demonstrated ticagrelor given at a maintenance dose of 90 mg bid reduced cardiovascular events in comparison with clopidogrel in ACS patients (20). The POPular AGE trial, involving patients in the ACS, ticagrelor, and prasugrel groups, showed just a 53% adherence rate during the 1-year follow-up, and this was in most part due to the side effects and recognized risk of bleeding events (46). The effect of ticagrelor on health outcomes in diabetes mellitus patient's intervention trial studied ticagrelor versus placebo in addition to aspirin in stable CAD patients with type 2 diabetes, a considerable 15% reduction in ischaemic events was observed with added ticagrelor (47). The ticagrelor 60 mg bid was studied to attain the same pharmacokinetic and pharmacodynamic effect as such of high dose as 90 mg bid in the prevention of cardiovascular events in patients with prior heart attack using ticagrelor compared to placebo on a background of aspirin–thrombolysis in myocardial infarction study (48). A long-term randomized clinical trial comparing standard antiplatelet therapy and individualized antiplatelet regimen based on the pharmacogenetic profile of acute ischemic minor stroke (AIMS) and transient ischemic stroke (TIA) patients in a Chinese population was undertaken to establish evidence to support the importance of genomic profiling to select P2Y12 receptor antagonists in such patients (49).

ANTIPLATELET RESISTANCE

Antiplatelet therapy is crucial to the secondary prevention of acute ischemic stroke to prevent Recurrent Ischemic Stroke (RIS) attacks (4). Despite its effectiveness and the proper intake of drugs, to some extent, aspirin or clopidogrel fail to produce pharmacological action, i.e., when it fails to inhibit platelet aggregation due to a reduction in platelet sensitivity and thus leads to recurrent adverse vascular events and this phenomenon led in coining the term "Resistance," which is now clinically referred as "High on Treatment Platelet Reactivity (HTPR)": the treatment failure of antiplatelet therapy (4–6, 50). Low or non-responders to antiplatelet treatment are more prone to resistance and are prone to increased risk of suffering RIS events and early neurological deterioration (6, 51, 52).

The different approaches used in defining antiplatelet resistance are (1) laboratory resistance—an increase in the levels of thromboxane A2 (TXA2) metabolites due to the inadequate inhibition of TXA2 and platelet aggregation despite antiplatelet therapy (53–55)—and (2) clinical resistance—when there is antiplatelet treatment failure (i.e., a failure to prevent antithrombotic event occurrence in stroke patients) (6, 53, 54). The most important factors for antiplatelet resistance in patients with AIS are due to poor adherence and concurrent use of other cyclooxygenase- 1 (COX- 1) inhibitors (56) and genetic factors like single nucleotide polymorphism (SNP) of the receptors ($P2Y_{12}$, $P2Y_1$, $GPIIb - IIIa$, collagen receptor, TXA2, etc.) and enzymes (COX-1&2). Other causes for resistance include the pharmaceutical preparation, anion efflux pump, interaction of platelets with other cells like endothelial cells or monocytes, accelerated platelet turnover, and activation of an alternate pathway for metabolism (57). Metabolic syndromes like diabetes mellitus because of hyper glycation of platelet protein but prediabetes is independent of resistance (56, 58, 59) hypercholesterolemia, increased body weight (obesity) (60, 61) smoking (62), and interaction with some drugs like Proton Pump Inhibitors (PPIs), e.g., esomeprazole and clopidogrel, and Non-Steroidal Anti-Inflammatory Drugs (NSAIDS), e.g., Ibuprofen and Aspirin (50, 53–55, 57, 63, 64). Examples of antiplatelet resistance causes are shown in **Figure 1**.

A study on 69 patients on the prognostic value of high platelet reactivity in ischemic stroke depending on etiology based on large- and small-vessel disease concluded that large vessel disease worsens early prognosis and in small vessel disease worsens late prognosis and clinical and functional condition of the patients, thus resistances is also dependent on the etiology of the stroke condition (65). This was confirmed in a 3-year follow-up period study where they also concluded that there is the large-vessel etiology of AIS is associated with the occurrence of adverse vascular events in HTPR patients and it is also associated with large infarct volume in the patients (66) and HTPR also leads in the formation of ischemic lesions in the brain (67). A Cytokine Registry in Stroke Patients (CRISP) study conducted in India based on the response of clopidogrel resistance in ischemic stroke patients has linked female sex and proton pump inhibitors use rather than cytochrome polymorphism (68). In the Chinese population, it was found that clopidogrel resistance due to a polymorphism of the CYP2C19*2 allele with or without hypertension and a P2Y12 receptor variant (68, 69) is associated with recurrent ischemic stroke, adverse vascular events, and poor recovery from neurological deficits (70). Another study postulated that CYP2C19*2 allele polymorphism or loss of function of CYP2C19*3 are at high risk for clopidogrel resistance (71), and thus it can be assumed that the clopidogrel resistance is mostly due to CYP2C19 polymorphism which was conformed in systematic review and meta-analysis by Alakbarzade et al. (71). Therefore, the cause for resistance from antiplatelet therapy is multifactorial, and genetic polymorphisms play a major role in resistance etiology.

Platelet function guided antiplatelet therapy is getting more important because of increased resistance from antiplatelet drugs like aspirin and clopidogrel which is included in most AIS patients, and they experience different adverse vascular events due to the treatment failure. It also helps in the tailored or personalized antiplatelet therapy in the patients who have high on-treatment platelet reactivity and in the early detection of adverse vascular events (7, 8). So, it is important to measure the inhibition of the platelet function in patients with AIS who have HTPR (72). The different platelet function testing methods are bleeding time, light transmission platelet aggregation (LTA), impedance platelet aggregation, lumi-aggregometry, and tests based on platelet function methods combined with viscoelastic tests, such as Thromboelastographs (TEGs)/platelet mapping systems, Rotational Thromboelastometry (ROTEM) platelets, and others, where Flow Cytometry is used to test the platelet activation, and Radio- or Enzyme-Linked Immuno Assay measure the thromboxane A2 metabolites (8, 57, 73–75).

Despite the development of these many types of analyses to test the responsiveness of the antiplatelet therapy there remain several drawbacks, which ultimately create an upcoming challenge. The challenges faced during the Platelet Function Test (PFT)-guided antiplatelet therapy are due to the lack of consistency and standardization, automation, difficulty in the process, and inability to fulfill all the parameter needed in one test; it is also a promising challenge for researchers in making the assays into the clinical laboratory since most do not make through it (76, 77). The accuracy to capture the *in vivo* platelet function with *in vitro* platelet function test assays is still challenging (77). The other parameters reveal equipment that is expensive and time consuming to use in which a high volume of the sample is needed, and all the tests need well-trained staff to run the procedure. It is important to select the relevant test for the particular drug; it must be defined clearly. A study comparing PFT in AIS with antiplatelet therapy concluded that LTA-AA and TEG-AA showed a good correlation for monitoring the aspirin effect. PFA-EPI may be more likely to report resistance. TEG-ADP may not be appropriate for assessing platelet function in clopidogrel users. CYP2C19 genotyping will be the better option for the detection of platelet function (78). Nevertheless, different studies showed different results: a systematic review and meta-analysis of 1,136 participants included two retrospective studies based on platelet function analysis (PFA)-guided antiplatelet therapy in recurrent stroke with or without antiplatelet therapy modified (ATM) actions (79–81). Although there are many challenges, the PFT plays a vital role in the personalized antiplatelet therapy and the prediction

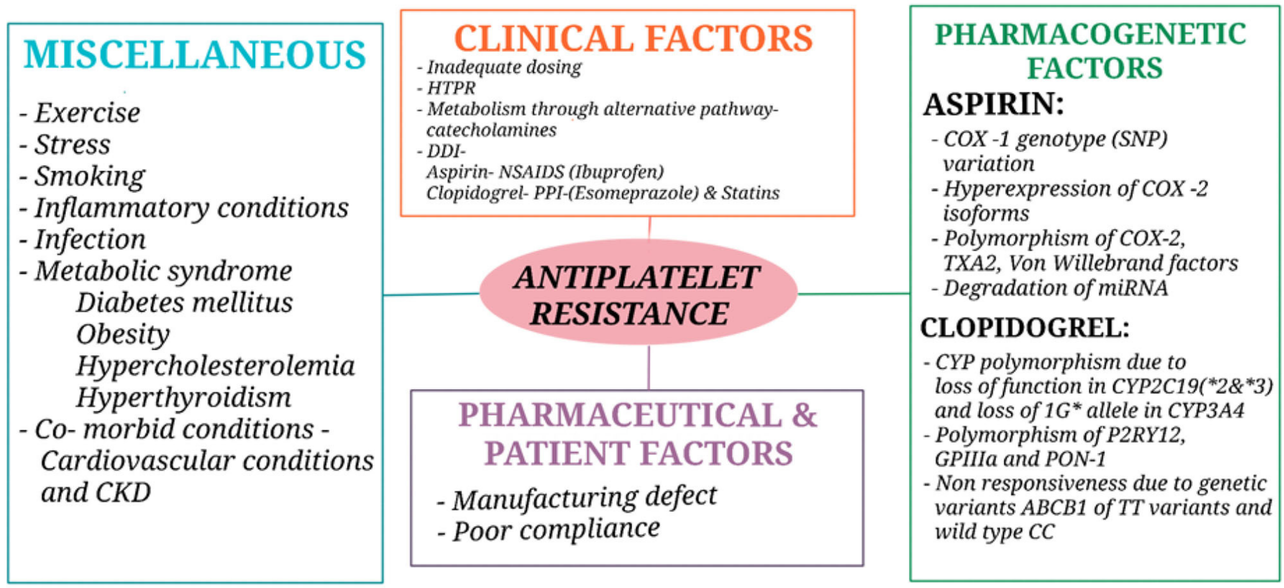

FIGURE 1 | Causes of antiplatelet resistance. CKD, Chronic kidney disease; HTPR, High on-treatment platelet reactivity; DDI, Drug drug interaction; NSAIDS, Non-steroidal anti-inflammatory drugs; PPI, Proton pump inhibitors; COX, Cyclooxygenase; TXA2, Thromboxane A2; miRNA, micro Ribonucleic acid; CYP, Cytochrome; GP, Glycoprotein; PON-1, Paraoxonase 1.

of early occurrence of bleeding and adverse vascular events in AIS patients.

Strategies for Identifying Aspirin Resistance

AR is a multifactorial pathological condition that has many different causes. The aspirin resistance can be identified both clinically and through laboratory methods. Clinically, it can be identified from the occurrence of atherothrombotic events in a patient who is under the therapeutic effect of one dose of aspirin. But this method is limited because it is mostly non-specific and can only be identified retrospectively because the events occur only after the start of the treatment (82, 83). The laboratory monitoring of PFT is based on the platelet aggregation and presence of platelet reactivity which is mentioned above. These PFTs are the most used methods for the detection of aspirin resistance. Despite its limitations, PFT is most specific and considerable over time (84). Aspirin resistance can be relevant with the prediction of concentration of proteinuria in patients with AIS, and these are on aspirin therapy. Thus, proteinuria can be considered as a tool in identifying aspirin resistance (11), and AR is useful as a prognostic marker for cardiovascular disorders and other comorbidities of AIS (85).

PHARMACOGENETIC VARIANTS OF ANTIPLATELET AGENTS

Pharmacogenetics of Aspirin

Multiple factors contribute to lowered aspirin efficacy (86) with genetic determinants attribute to 30% of cases (87). The patients with C765G (rs20417) polymorphism of COX-2 was established to have lowered risk of adverse cardiovascular events in aspirin users (Odds Ratio (OR): 0.78, 95% CI: 0.70– 0.87) (88). The PlA1/A2 SNP of the GPIIIa receptor gene was studied to be associated with lowered aspirin response. The SNP rs5918 in the ITGB3 gene was significantly associated with an amplified platelet response to aspirin (89).

Pharmacogenetics of Clopidogrel

Clopidogrel is a widely prescribed drug for the prevention of recurrent ischemic events in patients with ACS or MI due to its efficacy and cost-effectiveness compared to other antiplatelet agents. It is most commonly used along with aspirin as dual antiplatelet therapy in the prevention of atherothrombotic events. However, wide variability occurs between patients in response to clopidogrel therapy, and some even present with clopidogrel resistance. The CYP2C19 polymorphisms are the most common and well-studied polymorphisms associated with clopidogrel response (90). In trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with Prasugrel–Thrombolysis in myocardial infarction 38 trial, ACS PCI patients with ATP Binding Cassette Subfamily B Member 1 (ABCB1) T-allele homozygotes had adverse cardiovascular events like recurrent stroke and MI (91). Numerous Loss-of-Function (LOF) variants in CYP2C19 affect antiplatelet response to clopidogrel. SNP rs4244285 of CYP2C19*2 (92)and SNP rs12248560 of CYP2C19*17 contribute to altered clopidogrel response (86). Although, earlier studies have established the minimal association between polymorphisms such as CYP1A2*1F and CYP2C9*2/3 and response to clopidogrel. The later studies have failed to replicate any significant association (86, 93). Through the pharmacogenomics of anti-platelet intervention (PAPI) study involving 566 subjects, the missense polymorphism (G143E, rs71647871) was demonstrated to affect clopidogrel drug response and reactivity (94). Patients with Paraoxonase 1 192Q-allele homozygotes had reduced clopidogrel response and lowered bleeding complications (HR = 0.4, 95% CI: 0.2–0.8, $P = 0.006$) (88). ABCB1 C3435T variant in PCI patients with homozygous T allele showed significantly lower levels of the drug and hence the antiplatelet activity (95). Recognizing the impact this has on drug metabolism, the clinical pharmacogenetics implementation consortium (CPIC) guideline recommends alternate antiplatelet treatment for ACS/PCI patients estimated to be altered metabolizers of the drug (90).

Pharmacogenetics of Prasugrel and Ticagrelor

Numerous studies have investigated the association of CYP450 variants in response to prasugrel. SNPs rs4244285 and rs12248560 of CYP2C19 were found to be significantly associated with a prasugrel response. However, no association was established in CYP2C9, CYP2B6, CYP3A4, or CYP1A2 variants related to prasugrel response (96). Ticagrelor is a next-generation P2Y12 inhibitor. It gets disintegrated to an equally effective primary active metabolite, AR-C124910XX via CYP3A4/5 metabolism (97, 98). A genome-wide association study was conducted to detect SNPs associated with Ticagrelor levels and response from the PLATO clinical trial (99). SNP rs56324128 in CYP3A4, rs62471956 SNP in CYP3A43, rs61361928 SNP in UGT2B7, and rs4149056 SNP in SLCO1B1 were significantly associated with decreased levels of ticagrelor plasma concentrations. SNP rs113681054 of the SLCO1B1 gene, CYP3A4*1, and CYP3A4*22 variants of CYP3A4 were significantly associated with increased plasma ticagrelor concentrations. SNP rs4661012 in Platelet Endothelial Aggregation Receptor-1 (PEAR1) gene was associated with decreased ticagrelor response and SNPs-rs12566888 & rs12041331 in PEAR1 gene was associated with increased ticagrelor response. Where, CYP3A4*1, CYP3A4*22 variants are related to high inhibition of platelet aggregation (100–102). In **Table 2**, the association between a pharmacogenetic variant and a drug phenotype is summarized.

BIOMARKERS IN ACUTE ISCHEMIC STROKE

Numerous types of biomarkers are investigated in stroke, including physical, imaging, histological, genetic, electrophysiological, neuronal, and serum markers. Among these, genetic biomarkers can aid in personalizing stroke management through the detection of genetic variations including heritable cerebrovascular disorders. The Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification based on clinical parameters is the currently used method of ischemic stroke classification (114–116). Stroke occurrence is multifactorial with various mechanisms involved in its different subtypes. The development of specific novel and reliable biomarkers will be of great clinical significance. Platelets play a vital role in hemostasis. The human genome is estimated to encode around 1000 miRNAs. More than 100 of these are detected in human sera of healthy individuals and are termed circulating miRNAs (117). miRNAs, endogenous non-coding RNA molecules, are found to be abundant in platelets and are studied to be associated with platelet activity, inhibition, and responsiveness, making them good candidates as biomarkers. They inhibit mRNA translation and are released from platelets upon activation. Several studies have proposed the use of miRNAs as potential biomarkers to study platelet response in patients receiving antiplatelet treatment throughout the course of therapy as it plays a vital role in pathophysiological processes of stroke-related injuries. miRNAs and their target genes are involved in a variety of ischemic stroke pathophysiologies, including angiogenesis and neurogenesis (118). miRNAs are found to target many proteins in various regulatory cell signaling loci and signaling pathways in platelets. Several miRNAs play roles in both intrinsic and extrinsic apoptosis pathways. In the extrinsic apoptosis pathway, miR-21 and miR-25 are found to regulate TNF-α signaling affecting the stroke outcome. Upregulation of miR-155 reduces inflammation via miR-155–CARHSP1–TNF-α signaling (119). As a result, miRNA profiling appears to be a promising diagnostic marker for ischemic stroke in the future. miR-223, let-7c, and miR19a are the most copious platelet miRNAs. Reduced levels of miRNAs like miR-191, miR-126, miR-150, and miR-223 were detected in the plasma of healthy subjects treated with increasing dose of aspirin with prasugrel, indicating miRNAs response to platelet inhibition (120). Similarly, in healthy individuals treated with clopidogrel and ticagrelor, reduced levels of miR-223* and miR-197 were observed (121). The miR-96, miR-107, miR-200b, miR-223 and miR- 495 are significantly associated with platelet activation, secretion, and reactivity (1). miR-128b, miR-124, and miR-1246 have been studied to be associated with ischemic stroke and are detected to be up-regulated in stroke patients compared to healthy subjects (122, 123). In ischemic stroke patients with infarcts >2 cm^3, the elevated levels of miRNAs like miR-9-5p, miR-9-3p, miR-124-3p, and miR-128-3p were detected through next-generation sequencing technology indicating release of miRNAs with injury (114).

In patients of T2DM with ischemic stroke, the platelet miR-144 level was found to be elevated, while levels of platelet miR-223 and miR-146a were reduced (124). Significant reductions in levels of plasma miRNAs- miR-223, miR-126, and miR-150 were observed in patients treated with more potent antiplatelet agents such as P2Y12 inhibitors (125). Jager et al. (126) in a study on miRNAs- miR-223, miR-150, miR-126, and miR-21 established to be related to platelet function, suggested that these miRNAs may not be used as platelet activation related biomarkers after cessation of P2Y12 inhibitors treatment. Tiedt et al. (127) in their comprehensive study, identified three circulating miRNAs, 125a-5p, 125b-5p, and 143-3p, as potential biomarkers after acute ischemic stroke. Neutrophil extracellular traps (NETs) were detected in plasma and thrombus of ischemic stroke, suggestive a new prognostic biomarker in acute ischemic stroke patients (128, 129).

TABLE 2 | Pharmacogenetic variant association of antiplatelet drugs.

GENE	Ref SNP (rs) number	Association	Condition	Population	References
CYP3A4	rs56324128	Genotype CC is associated with reduced levels of ticagrelor compared to genotype CT.	ACS	European	(101)
SLCO1B1	rs113681054	Allele C in comparison with allele T is associated with elevated ticagrelor levels.	ACS	European	(101)
	rs4149056	Allele T compared to allele C is associated with reduced levels of ticagrelor.	ACS	European	(101)
CYP3A43	rs62471956	Allele G is associated with reduced levels of ticagrelor as compared to allele A.	ACS	European	(101)
UGT2B7	rs61361928	Genotype TT is associated with reduced levels of ticagrelor as compared to genotype CT.	ACS	European	(101)
PEAR1	rs12566888	Genotype TT is associated with elevated response to ticagrelor as compared to genotype GT.	Healthy individuals	Chinese	(102)
	rs4661012	Genotypes GT + TT is associated with reduced response to ticagrelor as compared to genotype GG.	Healthy individuals	Chinese	(102)
	rs12041331	Genotype AA is associated with augmented response to ticagrelor as compared to genotypes AG + GG.	Healthy individuals	Chinese	(102)
	rs12041331	Genotype AA is associated with increased response to ticagrelor as compared to genotype GG.	Healthy individuals	Chinese	(102)
P2RY1	rs1065776	Patients with genotype CT may have elevated risk of aspirin-resistant phenotype as compared to patients with genotype TT.	CAD	European	(103)
		Patients with genotype CT may have reduction in AA-induced platelet aggregation after aspirin treatment as compared to patients with genotype CC.	Healthy individuals	Chinese	(104)
ITGB3	rs5918	Patients with genotype TT may have aspirin-depressed thrombin generation and prolonged bleeding time after aspirin treatment as compared to patients with genotypes CC + CT.	CAD	Poland	(105)
		Patients with genotypes CC + CT may possess elevated risk of lack of aspirin response as compared to patients with genotype TT.	CAD	Poland	(106)
		Patients with genotype TT may have elevated risk of inadequate inhibition of platelet activity as compared to patients with genotypes CC + CT.	CAD	Tunisian	(107)
		Patients with genotype CT may have reduced aspirin mediated platelet inhibition as compared to patients with genotype TT.	CAD	United States	(89)
LPA	rs3798220	Patients with genotype CT may have reduced risk of Myocardial Infarction on aspirin treatment.	Healthy individuals	European	(108)
TBXA2R	rs4523	Patients with genotype AA may have elevated risk of residual platelet reactivity with aspirin treatment as compared to patients with genotypes AG + GG.	Off-pump coronary artery bypass grafting	Chinese	(109)
GP6	rs1613662	Patients with genotype AG may have elevated risk of non-response to aspirin as compared to patients with genotype GG.	CAD	Finland	(110)
GP1BA	rs6065	Patients with genotypes CT + TT may have elevated response to aspirin in men as compared to patients with genotype CC.	Healthy individuals	Japan	(111)
CYP2C19	rs4244285	Patients with allele A may possess an elevated risk of platelet reactivity as compared to patients with genotype GG.	ACS	France	(112)
		Patients with allele A may have increased platelet reactivity index (PRI) vasodilator-stimulated phosphoprotein (VASP) at 1 month of prasugrel treatment as compared to patients with genotype GG.	ACS	France	(112)
	rs12248560	Patients with allele T may have reduced platelet reactivity index (PRI) vasodilator-stimulated phosphoprotein (VASP) at 1 month of prasugrel treatment as compared to patients with genotype CC.	ACS	France	(112)
		Patients with allele T may have a reduced rate of high on-treatment platelet reactivity (HTPR) at 1 month of prasugrel treatment as compared to patients with genotype CC.	ACS	France	(112)
		Patients with allele T may possess escalated rate of hyper-response at 1 month of prasugrel treatment as compared to patients with genotype CC.	ACS	France	(112)

(Continued)

TABLE 2 | Continued

GENE	Ref SNP (rs) number	Association	Condition	Population	References
PEAR1	rs41273215	Patients with genotype TT may have reduced levels of inhibition of ADP-induced platelet aggregation compared to patients with genotypes CC + CT.	Healthy individuals	Chinese	(113)
	rs3737224	Patients with genotype TT may have reduced levels of inhibition of ADP-induced platelet aggregation compared to patients with genotypes CC + CT.	Healthy individuals	Chinese	(113)
	rs77235035	Patients with genotype AA may have reduced levels of inhibition of ADP-induced platelet aggregation as compared to patients with genotypes AC + CC.	Healthy individuals	Chinese	(113)
	rs822442	Patients with genotype AA are associated with reduced levels of inhibition of ADP-induced platelet aggregation as compared to patients with genotypes AC + CC.	Healthy individuals	Chinese	(113)
	rs822441	Patients with genotype CC are associated with reduced levels of inhibition of ADP-induced platelet aggregation as compared to patients with genotypes CG + GG.	Healthy individuals	Chinese	(113)
	rs12407843	Patients with genotype AA are associated with reduced inhibition of ADP-induced platelet aggregation as compared to patients with genotypes AG + GG.	Healthy individuals	Chinese	(113)

CYP3A4, Cytochrome P450 Family 3 Subfamily A Member 4; SLCO1B1, Solute Carrier Organic Anion Transporter Family Member 1B1; CYP3A43, Cytochrome P450 Family 3 Subfamily A Member 43; UGT2B7, UDP Glucuronosyltransferase Family 2 Member B7; P2RY1, Purinergic Receptor P2Y1; ITGB3, Integrin Subunit Beta 3; LPA, Lipoprotein(A); TBXA2R, Thromboxane A2 Receptor; GP6, Glycoprotein VI Platelet; GP1BA, Glycoprotein Ib Platelet Subunit Alpha; CYP2C19, Cytochrome P450 Family 2 Subfamily C Member 19; PEAR1, Platelet Endothelial Aggregation Receptor 1.

Numerous evidence from past studies has established the relationship between mean platelet volume (MPV) and cerebrovascular events (130, 131). Some suggested the use of mean platelet volume (MPV) as a potential diagnostic and prognostic biomarker of acute ischemic stroke (132). In certain studies, MPV was detected to be raised both in acute ischemic stroke and certain hemorrhagic strokes (133). The range of MPV and MPV/Platelet count (PC) ratio was studied to be significantly represented in stroke patients than healthy individuals (134, 135). Also the MPV and MPV/PC ratio tests are cost-effective, relatively simple, and can aid risk identification of stroke (136). Along with that, MPV levels are suggested to vary among stroke subtypes depending on the severity of injury and size of the infarct. The levels of MPV and MPV/PC ratio were studied to be significantly higher in atrial fibrillation (AF) stroke than large artery atherosclerosis (LAA) stroke, where both are subtypes of ischemic stroke (137). Hence, it can act as a biomarker in stratifying the stroke subtypes and severity and as a prognostic metric of secondary stroke occurrence (138, 139). Conversely, some have failed to replicate the association in their studies. Although those studies are presented with several limitations (140).

Eventually, extracellular vesicles (EVs) and their molecules are being investigated as biomarkers in stroke pathogenesis and in stratifying stroke subtypes (141). Platelet activation triggers the release of EVs. It is classified into three types based on their size and source: microvesicles, exosomes, and apoptotic bodies. It is regulated by the MISEV2018 guidelines recommended by "The International Society for Extracellular Vesicles (ISEV)" (142). Circulating EVs released from platelets stimulate endothelial cells and vascular smooth muscle cells, increasing vascular tissue inflammation and repair. The immunomodulatory role of platelet-derived EVs on CD4+ T cells in promoting platelet and fibrin aggregation and adhesion on vessel walls increases the risk of thrombus formation (143). Circulating EVs are elevated in patients with ACS and atherothrombotic incidents, especially in the initial hours of the event.

DISCUSSION

Currently, stroke management largely relies on empirical antiplatelet therapy, though many populations exhibit wide potential genetic variations leading to therapeutic failure, presenting with treatment complications and recurrent thrombotic events. Various genetic determinants of antiplatelet agents- aspirin, clopidogrel, prasugrel, and ticagrelor have been identified. They were studied to be associated with antiplatelet therapy efficacy, response, adverse events, and toxicity. Reduced response to antiplatelet therapy in patients with genetic variants has been studied aiding in therapy optimization. For example, patients with PlA1/A2 SNP of the GPIIIa receptor gene were demonstrated to have decreased response to aspirin (144). Likewise, drug toxicity in patients has been detected. For example, patients with CYP2C19 gain of function variants receiving clopidogrel therapy have a high risk of presenting with bleeding complications. Similarly, patients with rs5050 of angiotensinogen (AGT) gene receiving aspirin showed an elevated risk of peptic ulcer hemorrhage especially with genotype GG (145). The Clinical Pharmacogenetics Implementation Consortium (CPIC) tried to compile such adverse events related to genetic data in clinical algorithms for clopidogrel aiding in therapy optimization (146). This necessitates the detection of more genetic variants associated with antiplatelet drugs. With the advancement of high-throughput sequencing technologies, whole-genome sequencing in many populations has become possible. Newer genetic associations with clopidogrel response were detected by Genotype Information and Functional Testing (GIFT) exome study, ATP2B2, and TIAM2 through whole-exome sequencing (147). The number of physical, genetic, serum, and plasma biomarkers related to ischemic stroke has been identified. Specific miRNAs were found to be altered before the stroke occurrence, and these could be used as diagnostic and predictive biomarkers of stroke.

The clinical translation of pharmacogenomics testing in stroke management in using appropriate antiplatelet therapy will prevent adverse thrombotic events while improving therapeutic outcomes. Many studies have established the importance of platelet function testing (PFT)-guided antiplatelet therapy (148, 149). PFT is found to be more cost-effective in detecting antiplatelet response in comparison with genomic sequencing technologies (150). However, guidelines on PFT- or genotype-guided antiplatelet treatment are not well established given the ambiguity in studies (151, 152). A recent comparative study on PFTs on ischemic stroke patients has demonstrated that light transmittance aggregometry arachidonic acid platelet agonist (LTA-AA) and thromboelastographic arachidonic acid platelet agonist (TEG-AA) are effective in monitoring aspirin efficacy and response (78). Dual antiplatelet therapy (DAPT), comprising clopidogrel and aspirin is an effective strategy in managing the recurrence of stroke-related events. The dual-antiplatelet therapy (DAPT) score was developed to predict ischemic and bleeding risk in patients treated with percutaneous coronary intervention (PCI) (153, 154). The DAPT score and its decision tool was validated by several other studies including a meta-analysis, which concluded that it is helpful in characterizing ischaemic and bleeding events risk in post PCI patients and helps in deciding the desired duration of DAPT treatment (155). Another validated score in predicting bleeding complications while using DAPT is the PRECISE-DAPT score. The correlative analysis of genotypic data with clinical phenotyping data and platelet function tests will be a promising futuristic goal. This was achieved by Dewey et al. in their study, through whole-exome sequencing 50,000 subjects (88). Studies have been conducted, undertaking personalized approach based identified genetic variants. In stable CAD patients of the Chinese population, personalizing antiplatelet treatment based on maximum aggregation rate (MAR) in comparison with standard DAPT improved the health outcome after 180-day follow-up after PCI (156). According to a meta-analysis conducted recently in patients presenting with high platelet reactivity (HPR), platelet function test-based intensification of DAPT led to a reduction in adverse events (157). As diversity in both genotype and phenotype exists across different population groups, along with the need to determine the appropriate therapy for each individual, personalized medicine is the most promising futuristic approach in managing complex cerebrovascular events like acute ischemic stroke.

CONCLUSIONS

The integration of specific biomarkers, genotype- as well as phenotype-related data in antiplatelet therapy stratification in patients with acute ischemic stroke will be of great clinical significance. However, the data on genetic determinants and biomarkers with specificity is limited. Ongoing and future clinical studies are hoped to yield further valuable evidence and standardized guidelines in translating a personalized approach to the management of ischemic stroke. This futuristic approach is believed to offer better management of thrombotic events while preventing stroke and antiplatelet drug-related complications.

AUTHOR CONTRIBUTIONS

PV, SP, and AA contributed to first draft of manuscript and acquisition of data. MM, MS, AA, and AK contributed to the analysis, interpretation, and critical revision of the manuscript for important intellectual content. MKS contributed to the literature review and critical revision. All authors contributed to the article and approved the submitted version.

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Novel Predictors of Future Vascular Events in Post-stroke Patients—A Pilot Study

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Introduction: A modified platelet function test (mPFT) was recently found to be superior compared to impedance aggregometry for selection of post-stroke patients with high on-treatment platelet reactivity (HTPR). We aimed to explore some peripheral blood cell characteristics as predictors of recurrent ischemic episodes. The predictive value of mPFT was also assessed in a cohort followed up to 36 months regarding recurrent ischemic vascular events.

Methods: As a novelty, not only whole blood (WB), but after 1-h gravity sedimentation the separated upper (UB) and lower half blood (LB) samples were analyzed including neutrophil antisedimentation rate (NAR) in 52 post-stroke patients taking clopidogrel. Area under the curve (AUC, AUC_{upper} and AUC_{lower}, respectively) was separately measured by Multiplate in the WB, UB and LB samples to characterize *ex vivo* platelet aggregation in the presence of ADP. Next, the occurrence of vascular events (stroke, acute coronary syndrome, ACS) were evaluated during 36-month follow-up.

Results: A total of 11 vascular events (stroke $n = 5$, ACS $n = 6$) occurred during the follow-up period. The AUC_{upper} was significantly higher in patients with recurrent stroke compared to those with uneventful follow-up ($p = 0.03$). The AUC_{upper} with a cut-off value ≥70 based on the mPFT, was able to predict all stroke events ($p = 0.01$), while the total vascular events were independently predicted by NAR with a sensitivity of 82% and specificity of 88%.

Conclusions: A combination of NAR reflecting the inflammatory state and AUC_{upper} indicating HTPR may provide a better prediction of recurrent ischemic events suggesting a better selection of patients at risk, thus providing an individually tailored vascular therapy.

Keywords: recurrent stroke, vascular event, platelet function, platelet reactivity, outcome

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INTRODUCTION

Despite successful recanalization strategies either with thrombolysis or using endovascular treatments for acute ischemic stroke, the eventual outcome of patients is far from desirable (1). Among many factors, some peripheral blood cells may play a pivotal role in post-procedural microcirculatory alterations contributing to the outcome (2, 3). A higher incidence of recurrent cerebral ischemia was described in post-stroke patients with high on-treatment platelet reactivity (HTPR) (4, 5). Numerous tests assessing *ex vivo* platelet reactivity were used for identification of patients at risk for HTPR (6). However, the prevalence of HTPR was shown to vary depending on the definition and assay used (7). A modified platelet function test (mPFT) was recently found to be superior compared to conventional Multiplate Electrode Aggregometry for selection of post-stroke patients with HTPR (8).

Therefore, we aimed to explore some peripheral blood cell characteristics including platelets and neutrophils as predictors of recurrent ischemic episodes and factors contributing to the outcome. The predictive value of the mPFT as a point-of-care test (POCT) was also compared to conventional Multiplate Electrode Aggregometry in a cohort followed up to 36 months regarding recurrent ischemic vascular events.

MATERIALS AND METHODS

Subjects

The study protocoll was approved by the University of Pecs Clinical Centre Regional and Institutional Research Ethics Comittee (8). Written informed consent was obtained from each patient. A total of 52 patients (age: 66 ± 8 years, male: 31) on antiplatelet therapy (75 mg clopidogrel once daily) due to secondary stroke prevention were prospectively recruited into this study. The selected patients with previous anterior circulation large artery atherothrombosis were on regular medical check-up at the Outpatient Clinic of the Department of Neurology. Fasting venous blood samples were taken *via* a 21G peripheral venous canula from each patient and healthy subjects. Patients were instructed to take their daily clopidogrel at least 2 h prior to blood sampling. Exclusion criteria were acute infection and acute vascular events, such as acute ischemic stroke (AIS), transient ischemic attack (TIA), acute myocardial infarction (AMI), acute coronary syndrome (ACS), thrombocytopenia (platelet count <150G/l), congenital platelet abnormalities, congenital disorder of haemostatis (e.g., hemophilia), anemia and patients on medical therapy influencing blood coagulation (e.g., oral anticoagulants, novel oral anticoagulants, non-steroid antiinflammatory drugs). The comorbidities, medications and smoking status were also recorded. Besides, the baseline erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and total blood count were measured. Next, the incidence of vascular events (ACS and recurrent ischemic stroke) in the total study population was evaluated in a 36-month follow-up. ACS was defined by using the ACC/AHA guidelines (shortly: based on clinical history, ECG results, levels of cardiac markers, and the results of stress testing). Each recurrent ischemic stroke was confirmed by neuroimaging (CT or MRI). All patients with either ACS or recurrent ischemic stroke were presented at the Emergency Department and underwent a careful clinical evaluation then archived by an electronic database.

Blood Sampling

Venopuncture was performed from the cubital vein after short time strangulation of the arm with 21G BD vacutainer needle. The total blood count was measured after taking into vacutainers with EDTA (REF: 368856, 5.4md EDTA). Whole blood for platelet aggregometry was also taken into hirudin containing tube for Multiplate Electrode Aggregometry.

Platelet Antisedimentation Rate, Neutrophil Antisedimentation Rate

Modified whole blood gravity sedimentation technique was developed for studying platelet and neutrophil sedimentation properties (8). After 1-h gravity sedimentation, the upper and lower half of the venous blood column was separately removed from the EDTA sedimentation tube and transferred to another EDTA tube for further analysis. An automatic cell counter system (Sysmex XN 9000, Sysmex Co, Japan, 2017) was applied to measure the upward floating (ascending) and sinking (non-ascending) cells in the separated samples. Next, the platelet antisedimentation rate (PAR, %), leukocyte antisedimentation rate (LAR,%) and neutrophil antisedimentation rate (NAR, %) were, respectively, calculated based on the equation:

$$\frac{\text{cell count}_{\text{upper}} - \text{cell count}_{\text{lower}}}{\text{cell count}_{\text{upper}} + \text{cell count}_{\text{lower}}} \text{X100}$$

Multiplate Electrode Aggregometry

Platelet function test in the whole blood was perfomed from a hirudin containing tube with a Multiplate® Analyzer (Roche Diagnostics, Mannheim, Germany). Another hirudin containing tube was used for sedimentation, similarly to whole blood sedimentation in the EDTA-tube. After 1-h gravity sedimentation, the blood coloumn was devided into upper and lower samples. Platelet aggregometry was uniformly performed 60 min after blood sampling using adenosine diphosphate (ADP; 6.5 M) as agonist. As a novelty, not only whole blood, but after 1-h gravity sedimentation the separated upper and lower half blood samples were simultaneously analyzed in each post-stroke patient taking clopidogrel. Aggregation level was expressed as the area under the curve (AUC). AUC was calculated by a Multiplate® Analyzer using the product of aggregation unit (AU) × time (minutes) (9). After ADP stimulation, the normal aggregation range was expected as AUC: 53–122 according to the manufacturer (9). Based on the whole blood AUC, patients on clopidogrel were categorized as responder cases with AUC <53 and resistant cases representing HTPR with an AUC ≥53 (10).

Statistical Analysis

Data were evaluated using SPSS software package (Version 19.0, SPSS Inc, Chicago, USA). Categorical data were summarized by means of absolute and relative frequencies (counts and percentages). Quantitative data were presented as median and 25th−75th percentiles, as well as mean ± SD. The Kolmogorov-Smirnov test was applied to check for normality. Chi-square test for categorical data and Student-*t* test for continuous data were used for analysis of demographic and clinical factors. Non-parametric Mann-Whitney *U* test was used for not normally distributed parameters. Correlation analysis was performed calculating Spearman's correlation coefficient (rho). A *p*-value <0.05 was considered statistically significant.

TABLE 1 | Demography and clinical data of the total population, and comparison between patients without vs. with recurrent vascular events during 36-month follow-up.

	Total population $n = 52$	Uneventful $n = 41$	Vascular events $n = 11$	*p*-value
age	66 ± 8	66 ± 8	66 ± 9	0.937
male, *n*	34	26	8	0.564
hypertension, *n*	51	40	11	0.601
diabetes mellitus, *n*	14	10	4	0.427
smoking, *n*	11	9	2	1.000
ESR	12 (8–18)	10 (8–16)	18 (14–29)	0.063
CRP	1.9 (0.7–4.6)	1.8 (0.7–5.0)	2.2 (1.4–3.35)	0.614
PLT	224 (200–260)	224 (207–251)	243 (171–300)	0.805
PAR	67.9 (63.1–73.4)	67.8 (62.9–73.5)	70.0 (64.6–72.6)	0.614
WBC	6.8 (5.8–8.0)	6.6 (5.8–7.9)	7.4 (5.5–10.6)	0.420
LAR	35.7 (23.7–46.3)	36.2 (24.7–46.4)	34.4 (24.0–43.5)	0.806
neutrophil	61.8 (55.4–66.4)	62 (56–67)	58 (51–62)	0.317
NAR	−1.1 (−4.8–6.5)	0.9 (−3.9–7.2)	−5.2 [−6.8–(−4.7)]	0.001

Vascular events, recurrent stroke, and de novo acute coronary event; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; PLT, platelet; PAR, platelet antisedimentation rate; WBC, white blood cell; LAR, leukocyte antisedimentation rate; NAR, neutrophil antisedimentation rate. Data are presented as median and 25th−75th percentiles, except age as mean ± SD.

TABLE 2 | Area under the curve (AUC) in the whole blood, and AUC in the upper and lower samples after 1-h gravity sedimentation in the total population and comparison between uneventful vs. stroke + ACS as well as uneventful vs. recurrent stroke subgroups.

	Total population $n = 52$	Uneventful $n = 41$	Stroke + ACS $n = 11$	*p*-value
AUC	40.5 (27–53.5)	40 (27–54)	42 (32.5-44)	0.866
AUC_{upper}	56 (22.5–76.5)	51.5 (19.5–77.5)	65 (42–75.5)	0.247
AUC_{lower}	18 (13.5–22)	18 (14–23)	17 (13–20)	0.567
	Total population $n = 52$	**Uneventful $n = 41$**	**Stroke events $n = 5$**	***p*-value**
AUC	40.5 (27–53.5)	39 (27–53)	43 (42–44)	0.347
AUC_{upper}	56 (22.5–76.5)	49 (21–74)	77 (71–92)	*0.020*
AUC_{lower}	18 (13.5–22)	18 (14–22)	17 (11–19)	0.763

AUC, area under the curve measured by Multiplate analyzer; AUC_{upper}, AUC in the upper sample after 1-h gravity sedimentation; AUC_{lower}, AUC in the lower sample after 1-h gravity sedimentation.

TABLE 3 | Predictors of vascular events during 36-month follow-up.

	β	*p*-value	OR	95% CI	
age	−0.071	0.353	0.931	0.801	1.082
AUC	−0.046	0.320	0.955	0.871	1.046
AUC_{upper}	−0.083	0.031	1.086	1.007	1.171
NAR	−0.489	0.032	0.613	0.392	0.960

AUC, area under the curve measured by Multiplate analyzer; AUC_{upper}, AUC in the upper sample after 1-h gravity sedimentation; NAR, neutrophil antisedimentation rate; OR, odds ratio, 95%CI, 95% confidence interval. Binary logistic regression analysis.

RESULTS

A total of 52 convalescent ischemic stroke patients were prospectively enrolled into this pilot study. All patients have been previously suffered from large vessel occlusion. The demography and clinical data of the study population is summarized in **Table 1**. A total of 11 vascular events (stroke n=5, ACS n=6) occured during 36-month follow-up. Of the antisedimentation rate indices, only NAR showed significant difference between "uneventful" vs. "vascular events" groups (**Table 1**). It is noteworthy that no difference was observed between the baseline blood count parameters (platelet, leukocyte, neutrophil), while a trend-like difference was observed in the ESR (**Table 1**). The AUC in the whole blood, and in the upper and lower samples after 1-h gravity sedimentation in the total population, and also a comparison between uneventful vs. stroke + ACS as well as uneventful vs. recurrent stroke alone subgroups are shown in (**Table 2**). The AUC_{upper} was significantly higher in patients with recurrent stroke compared to those with uneventful follow-up ($p = 0.03$) (**Table 2**).

Independent Predictors

Based on ROC analysis, the AUC_{upper} with a cut-off value ≥70 measured by the mPFT was able to predict recurrent stroke events ($p = 0.01$) with the best sensitivity and specificity. Moreover, the total vascular events (stroke+ACS) was independently predicted by NAR with a sensitivity of 82% and specificity of 88% using a multiple regression analysis including relevant covariates (**Table 3**). Neither recurrent stroke nor ACS showed association with HTPR status defined by AUC>53 measured by the Multiplate in the whole blood.

Cut-Off Values of Predictors

The ROC curves of variables predicting recurrence of vascular events during follow-up are shown in **Figure 1**. In this cohort, NAR with a cut-off ≥ -0.431 independently predicted recurrence of total vascular events (stroke + ACS, $n = 11$) with a sensitivity of 82% and specificity of 88% during 36-month follow-up (Area: 0.847, $p = 0.002$, 95%CI: 0.703–0.992) (**Figure 1A**). Furthermore, ROC of platelet function test based on impedance aggregometry in the upper blood sample after 1-h gravity sedimentation revealed, that AUC_{upper} with a cut-off ≥ 70 predicted recurrent stroke with a sensitivity of 80% and specificity of 74% during 36-month follow-up (Area:0.813, $p = 0.023$, 95%CI:0.689–0.937) (**Figure 1B**). Finally, a more precise model was created, when a ROC analysis was performed with predicted probablity of the combination of NAR and PFT_{upper} (Area:0.881, $p = 0.001$, 95%CI:0.754–1.0) (**Figure 1C**).

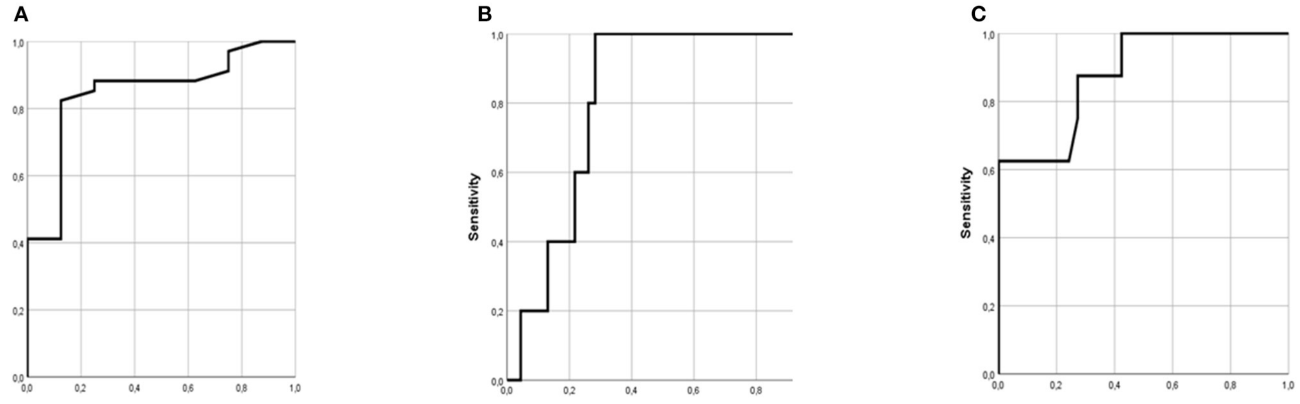

FIGURE 1 | ROC curves of variables predicting recurrence of vascular events during follow-up. **(A)** ROC of neutrophil antisedimentation rate (NAR) (Area: 0.847, p = 0.002, 95%CI: 0.703–0.992). **(B)** ROC of platelet function test based on impedance aggregometry in the upper blood sample (AUC_{upper}) after 1-h gravity sedimentation (Area: 0.813, p = 0.023, 95%CI: 0.689–0.937). **(C)** ROC of predicted probability of the combination of NAR and AUC_{upper} (Area: 0.881, p = 0.001, 95%CI: 0.754–1.0).

DISCUSSION

Activation of neutrophils reflected by NAR was shown here as the most sensitive marker of recurrence of ischemic cerebral episodes in post-stroke patients taking clopidogrel. Both, animal and clinical data support the pivotal role of activated peripheral blood cells (e.g., neutrophils, monocytes, platelets) in neuroinflammation due to ischemic stroke (2, 3, 11). One side, the dynamic microcirculatory stall phenomenon in the hyperacute stage can be a contributing factor to ongoing penumbral brain injury (2, 12), on the other side the sustained detrimental effects of activated leukocytes in the systemic circulation carries a constant risk in patients with chronic inflammatory state (e.g., vascular diseases) (13). Interestingly, a downward motion of neutrophils during 1-h gravity sedimentation expressed by a negative value of NAR was observed in those patients who suffered from composite vascular events during 36-month follow-up. In contrast, an upward motion of both, leukocytes and platelets proportionally to their activation was described previously in acute ischemic stroke (3), post-stroke infection (14) and burn patients (15). Neither LAR, nor PAR was found to be predictive for future vascular events in convalescent stroke patients suggesting that leukocytes and platelets exert their actions predominantly in the acute phase of stroke. Our finding also suggests that neutrophils are important markers of stroke outcome as their predictive role was recently shown in patients with acute coronary syndrome (16).

Numerous data highlight that a high proportion of patients with cardiovascular diseases have *ex vivo* HTPR on their prescribed antiplatelet regimen (4, 5, 7). Although several studies show an increased rate of recurrent cerebrovascular ischemic events in patients presenting HTPR, the diagnostics of HTPR has been unsolved so far (4, 17). Here, the state of clopidogrel resistance based on Multiplate electrode aggregometry from the whole blood was not able to predict recurrent stroke. However, a higher AUC ($\geq$70 as a cut-off value) from the separated upper blood sample after 1-h gravity sedimentation emerged as a novel independent predictor of future stroke episode in our study. This observation suggests that the upward motion of platelets might be associated with increased thrombotic tendency. Further studies are needed to explore the characteristics of this subpopulation of platelets and their impact on post-stroke complications and outcome. When the combination of NAR and PFT_{upper} was used in the statistical model, the predicted probability of a future vascular event was even more accurate.

In summary, while AUC_{upper} indicates more precise definition of HTPR, NAR rather reflects the inflammatory state in post-stroke patients (18). Based on this small, single-center pilot study, these novel markers may provide a better prediction of recurrent ischemic events leading to a better selection of patients at risk and providing an individually tailored vascular therapy including antiplatelet and anti-inflammatory regimens (17, 19).

LIMITATIONS

This is a small prospective cohort with a 36-month follow-up focusing primarily on recurrent coronary and cerebral ischemic episodes which required hospitalization. However, the silent ischemic lesion recurrence on MRI was not explored here. Therefore, a large, adequately sized, prospective multicenter study is needed to determine whether these novel assessments of HTPR in conjunction with pharmacogenetic and neuroimaging (diffusion weighted imaging, DWI) data, improves our ability to predict the risk of recurrent vascular events in patients with cardiovascular diseases. Although the interaction between inflammation and ischemic stroke is multifaceted, a better understanding of such mechanisms may lead to enhanced secondary prevention including immunomodulatory approaches and more precise antiplatelet therapy (20, 21).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Ethics Committee of the University of Pécs. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DS, EE, LS, and TM conceived, designed and coordinated the study, participated in acquisition, and interpretation of data. DS and TM drafted the manuscript. MT-F performed the laboratory measurements. MT-F and TM participated in the statistical analysis. All authors read and approved the manuscript.

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Clopidogrel Resistance in Patients With Stroke Recurrence Under Single or Dual Antiplatelet Treatment

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Background: The factors associated with clopidogrel resistance in patients with stroke recurrence receiving single or dual antiplatelet treatment (SAPT or DAPT) may differ. This study compared the high on-treatment platelet reactivities (HPRs) and the factors associated with clopidogrel resistance in recurrent ischemic stroke patients receiving clopidogrel or aspirin and clopidogrel.

Methods: We enrolled and allocated 275 recurrent ischemic stroke patients to the clopidogrel and DAPT groups and compared their demographics, conventional risk factors, and P2Y12 reaction units (PRUs). Clopidogrel resistance was categorized as PRU higher than 275. We performed a multivariate logistic regression analysis to determine the factors underlying clopidogrel resistance during SAPT and DAPT.

Results: In total, 145 (52.7%) and 130 (47.3%) patients received clopidogrel and DAPT, respectively at recurrence. The risk factors of the two groups were not significantly different, except that coronary artery disease was more frequent in the DAPT group. The PRU was higher (255 ± 91 vs. 221 ± 84; $p = 0.002$) and clopidogrel resistance was more frequent (45.5 vs. 31.5%; $p = 0.018$) in the SAPT than in the DAPT group. Hyperlipidemia was associated with clopidogrel resistance during SAPT, and smoking (Odds ratio = 0.426, 95% confidence interval 0.210–0.861; $p = 0.018$) had a protective effect against clopidogrel resistance. For those receiving DAPT, old age, female, low hemoglobin A1c level, and high ARU were associated with clopidogrel resistance.

Conclusions: HPR and clopidogrel resistance were more frequent in recurrent ischemic stroke patients receiving clopidogrel than in those receiving DAPT. Smoking was independently associated with less clopidogrel resistance among those receiving clopidogrel SAPT but not in those receiving DAPT.

Keywords: antiplatelet resistance, aspirin, clopidogrel, smoking, VerifyNow, prevention

INTRODUCTION

Antiplatelet treatment is one of the most important treatments for reducing non-cardioembolic ischemic stroke. However, a considerable proportion of patients still experience ischemic stroke recurrence during appropriate antiplatelet treatment. Various factors are involved in antiplatelet treatment failure. Approximately 20–30% of patients receiving antiplatelet treatment show high platelet reactivity (high on-treatment platelet reactivity; HPR) (1). Several factors affect HPR during clopidogrel treatment, including genetic variations and drug-drug interactions involving hepatic cytochrome P450.

As clopidogrel is a prodrug activated by the hepatic cytochrome P450, factors influencing the hepatic cytochrome P450 system may affect its response. Smoking, one of the major risk factors for ischemic stroke (2), also enhances the activity of the P450 system (3), which increases the efficacy of clopidogrel (smoker's paradox) (4, 5). Recently, a *post-hoc* analysis of the CHANCE (Clopidogrel in High-Risk Patients with Acute Non-Disabling Cerebrovascular Events) trial revealed the interaction between smoking status and the contribution of clopidogrel to the early recurrence of ischemic stroke (6). The incidence of stroke was lower in currently smoking than in non-smoking patients receiving treatment with aspirin and clopidogrel (dual antiplatelet treatment; DAPT).

However, the exact mechanism underlying the smoker's paradox observed in the previous study and the effect of smoking on the long-term use of clopidogrel in ischemic stroke patients was not verified. Furthermore, it is still unclear whether HPR is equally important in patients receiving long-term clopidogrel single antiplatelet treatment (SAPT) and DAPT (aspirin and clopidogrel). Here, we compared the HPR among patients who received clopidogrel SAPT and DAPT. The factors associated with clopidogrel resistance in recurrent ischemic stroke patients receiving clopidogrel SAPT and DAPT were investigated.

MATERIALS AND METHODS

Patients

This was a retrospective study involving consecutively registered patients with acute ischemic stroke (within 7 days from stroke onset) confirmed by magnetic resonance imaging (MRI). All the patients were admitted to Kyung Hee University Hospital and Jeonbuk National University Hospital between January 2010 and December 2017. Patients who were receiving clopidogrel or aspirin and clopidogrel at the onset of the stroke due to a prior ischemic stroke were enrolled. The use of DAPT was based on the protocols of each center, and finally by the physicians' decision based on the risk of bleeding and recurrence of ischemia. Usually, DAPT is used for a short period after ischemic stroke, whereas for (1) those with concomitant coronary artery disease (CAD), (2) severe intra or extracranial cerebral artery stenosis, or (3) recurrent cardiovascular event under SAPT, DAPT was considered for a longer-duration. The duration of DAPT was also determined based on the physician's decision. Patients without clinical (i.e., history of prior use of antithrombotics unobtainable) or imaging (inappropriate to receive MRI) data and those without the results of VerifyNow tests were excluded.

All those who were admitted to the two centers and were receiving clopidogrel or aspirin and clopidogrel at the onset of stroke underwent routine VerifyNow P2Y12 tests or VerifyNow Aspirin and VerifyNow P2Y12 tests, respectively, on the day of admission to investigate the biochemical antiplatelet resistance. The use and adherence of any antithrombotics were investigated from the patient, caregiver or physicians prescribing any medication prior to stroke.

Data Collection and Definition

We obtained the clinical and imaging data from a registry database and medical records, and we divided the enrolled patients into clopidogrel and DAPT (aspirin and clopidogrel) groups according to the antiplatelet treatment they received at the onset of the ischemic stroke recurrence. We investigated the factors associated with clopidogrel resistance in those receiving clopidogrel alone or in combination with aspirin. The patients who were smoking at the time of the study were categorized as smokers, whereas those who were not smoking or had stopped smoking for more than 1 year were categorized as non-smokers. We also reviewed the results of the laboratory tests and physical examination. These were results for hypertension, diabetes mellitus (DM), hyperlipidemia, and CAD, among others, which are the putative risk factors of cerebrovascular disease. Hypertension was defined as a case where 140/90 mmHg or more was found if it was checked while resting during admission. Hypertension was diagnosed by a previous history or measuring blood pressure after taking a break for 5 min or more when checking it at the hospital before discharge after the patients was stabilized. In case of suspicious white coat hypertension, the patient was recommended to write a blood pressure diary at home, and was considered at the first visit. DM was defined as a blood glucose level of >200 mg/dL for at least 2 h after an oral glucose challenge, fasting blood glucose level of >126 mg/dL, hemoglobin A1c (HbA1c) > 6.5%, or DM medication use (7). Hyperlipidemia was defined as venous low-density lipoprotein (LDL) cholesterol concentration of >160 mg/dL, total cholesterol (TC) of >240 mg/dL, and triglyceride (TG) >200 mg/dL (8). All three definitions were based on the levels after more than 12 h of fasting. CAD was established by CAD diagnosis by a cardiologist and CAD medication use or history of percutaneous coronary intervention or bypass surgery. The institutional review board of Jeonbuk National University Hospital approved this study (approval number: CUH 2020-01-008). We carried out all the procedures following the ethical standards of the institutional and national research committees and the Helsinki Declaration. Informed consent was waived due to the retrospective nature of the study.

VerifyNow Aspirin and P2Y12 Assays

We used the VerifyNow P2Y12 assays to measure the aspirin reaction unit (ARU), the P2Y12 reaction unit (PRU) and the percentage inhibition of the platelet P2Y12 receptors. This method is based on the ability of activated platelets to bind to fibrinogen. It measures the changes in light transmittance to assess fibrinogen-mediated platelet aggregation in blood containing clopidogrel (9). The degree of aggregation is expressed as ARU for aspirin and PRU and the inhibition percentage for clopidogrel. A higher ARU value reflects greater arachidonic acid-induced platelet reactivity, and a higher PRU value reflects greater ADP-induced platelet activity. An ARU equal to or higher than 550 is defined as aspirin resistance. Because of the high prevalence of the CYP 2C19 variant in Korea, a PRU higher than 275 was predictive of clinical events. Therefore, in this study, a PRU higher than 275 was considered indicative of clopidogrel resistance (10–13).

Statistical Analysis

First, we compared the demographics, clinical data, and HPR of the patients receiving clopidogrel and DAPT. We used Pearson's chi-squared or Fisher's exact test for categorical variables and student's *t*-test for continuous variables. The normality of distribution was tested and variables not showing normal distribution were test with Mann-Whitney *U* test and was presented as mean and interquartile ranges. Second, we performed a multivariate analysis to determine the independent factors associated with clopidogrel resistance in patients who received clopidogrel alone or in combination with aspirin. To avoid variable selection caused by spurious correlations, we included only the variables that were potentially associated with clopidogrel resistance ($p < 0.1$) on univariate analysis as potential factors associated with clopidogrel resistance for the multivariate logistic regression model. Factors associated with PRU were also investigated using a multivariable analysis with linear regression model. The correlations between age and the ARU and PRU levels were investigated using the Pearson correlation coefficient. We set statistical significance at $p < 0.05$ (two-tailed). We used SPSS 21.0 (IBM Corporation, Armonk, NY) to perform all the statistical analyses.

RESULTS

In total, 7,183 patients with onsets of acute ischemic strokes and transient ischemic attacks (TIA) within the previous 7 days were hospitalized and registered in the database. After excluding those with TIA (689 patients) and first stroke experience (4,879 patients), we identified 1,615 participants as recurrent ischemic stroke patients. Of them, 492 patients were taking aspirin and 362 were taking other antiplatelet agents or not taking an antiplatelet agent. Additionally, we excluded patients who did not have MR image or with poor image quality ($n = 198$), had limited clinical data ($n = 96$) and patients without VerifyNow data ($n = 192$). Consequently, the study evaluated the data of 275 recurrent ischemic stroke patients (**Figure 1**). The mean age of the enrolled patients was 70.2 ± 10.2 years-old, and 166 (60.4%) of them were males.

HPR Among the Clopidogrel and DAPT Groups

The subjects were treated with clopidogrel ($n = 145$; 52.7%) or DAPT ($n = 130$; 47.3%) at the time of stroke recurrence. There were no significant differences between the demographics or risk factors of the two groups, except that the prevalence of previous CAD was higher in those receiving DAPT (50.6%) than in those receiving clopidogrel (23.9%). Patients taking clopidogrel showed significantly higher PRU values than those in the DAPT group (255 ± 91 vs. 221 ± 84; $p = 0.002$). The proportion of patients with clopidogrel resistance was also higher in those with recurrent stroke using clopidogrel than those using DAPT (45.5 vs. 31.5%; $p = 0.018$, **Table 1**).

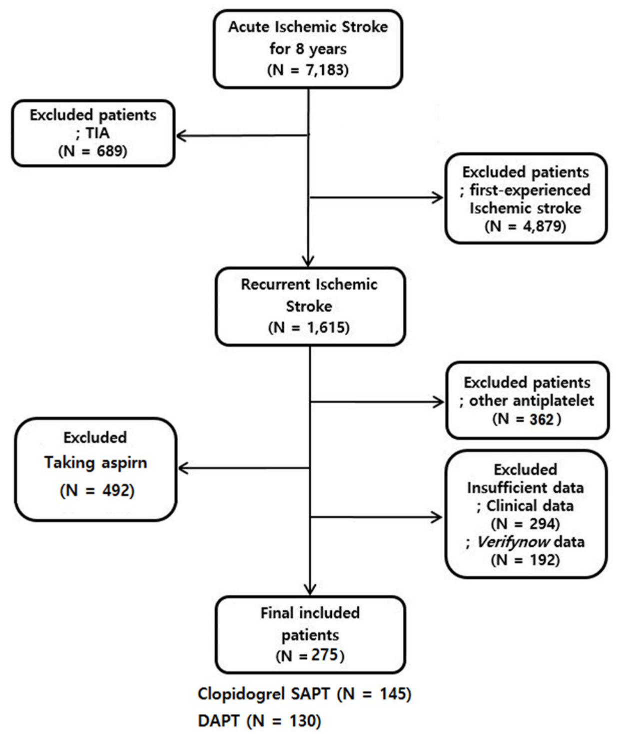

FIGURE 1 | Selection of recurrent ischemic stroke patients who received clopidogrel or DAPT. TIA indicates a transient ischemic attack.

Factors Associated With Clopidogrel Resistance in Clopidogrel SAPT

Among 145 patients who had recurrent ischemic stroke and were receiving clopidogrel SAPT, 66 (45.5%) showed clopidogrel resistance. Those with clopidogrel resistance had a higher prevalence of hyperlipidemia ($p = 0.018$). The prevalence of smoking was lower in those with than in those without clopidogrel resistance ($p = 0.022$). The multivariate analysis revealed that hyperlipidemia was associated with clopidogrel resistance (odds ratio [OR] = 2.625, 95% CI = 1.187–5.805; $p = 0.017$). Smoking had a protective effect against clopidogrel resistance (OR = 0.426, 95% CI 0.210–0.861, $p = 0.018$, **Table 2**).

A history of CAD (beta = 0.119; 0.194–0.672; $p = 0.001$) and smoking (beta = −0.315, −0.490–−0.081; $p = 0.007$) was independently associated with a high PRU value.

TABLE 1 | Clinical characteristics and HPR stratified by antiplatelet treatment for stroke recurrence.

	Clopidogrel ($n = 145$)	DAPT ($n = 130$)	*P-value*
Age, years	69.7 (10.9)	70.7 (9.4)	0.410
Male	87 (60.0)	79 (60.8)	0.896
Hypertension	131 (90.3)	108 (83.1)	0.074
Diabetes mellitus	68 (46.9)	63 (48.5)	0.795
Hyperlipidemia	102 (71.3)	103 (79.2)	0.132
Smoking	61 (42.1)	56 (43.1)	0.866
History of CAD*	16 (23.9)	40 (50.6)	0.001
Body-mass index (Kg/m^2)	25 (3.8)	24 (3.5)	0.335
Laboratory results			
C-reactive protein (mg/L)	0.14 [0.04–0.40]	0.23 [0.05–0.73]	0.153
Hemoglobin A1c (%)	6.6 (1.4)	6.9 (1.6)	0.082
Antiplatelet resistance			
Aspirin reaction unit	NA	444 (75)	NA
P2Y12 reaction unit (base)	293 (64)	291 (61)	0.762
P2Y12 reaction unit	255 (91)	221 (84)	0.002
Percent inhibition	13 (26)	22 (29)	0.003
Clopidogrel resistance (%)	66 (45.5)	41 (31.5)	0.018
NIHSS score (initial)	4 (3–5)	4 (2-5)	0.155
NIHSS score (discharge)	3 (2-5)	3 (1-4)	0.003
mRS (discharge)	2 (1-3)	2 (1-3)	0.289

Results are expressed as number (% column), mean (SD), or median (25–75 percentile range).
Non-parametric test was performed for continuous variables not showing normal distribution and presented as median (25–75 percentile range).
HPR, high on-treatment platelet reactivity; DAPT, dual antiplatelet treatment; CAD, coronary artery disease; NIHSS, National Institute of Stroke Scale; mRS, modified Rankin Scale.
**History of CAD: Clopidogrel (n = 67), DAPT (n = 79).*

TABLE 2 | Factors associated with clopidogrel resistance after clopidogrel SAPT.

	Clopidogrel Resistance (–) ($n = 79$)	Clopidogrel Resistance (+) ($n = 66$)	*P-value*	OR*	95% CI	*P-value*
Age, years	70.0 (10.3)	69.3 (11.6)	0.712			
Male	49 (62.0)	38 (57.6)	0.586			
Hypertension	72 (91.1)	59 (89.4)	0.723			
Diabetes mellitus	34 (43.0)	34 (51.5)	0.308			
Hyperlipidemia	50 (63.3)	52 (81.3)	0.018	2.625	1.187–5.805	0.017
Smoking	40 (50.6)	21 (31.8)	0.022	0.426	0.210–0.861	0.018
History of CAD	7 (8.9)	9 (13.6)	0.361			
BMI (Kg/m^2)	25 (4.0)	25 (3.6)	0.972			
Laboratory findings						
C-reactive protein (mg/L)	0.9 (2.4)	1.3 (2.9)	0.436			
Hemoglobin A1c (%)	6.6 (1.5)	6.5 (1.3)	0.766			

Results are expressed by number (% column), mean (SD), or median (25–75 percentile range).
**Factors entered to model: Dyslipidemia Smoking.*
CR, clopidogrel resistance; OR, odds ratio; CI, confidential interval; CAD, coronary artery disease; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; NIHSS, National Institute of Stroke Scale; mRS, modified Rankin Scale.

Factors Associated With Clopidogrel Resistance in DAPT

Among 131 recurrent ischemic stroke patients receiving DAPT, those with clopidogrel resistance ($n = 41$; 31.5%) were likely to be older (75 ± 8 vs. 69 ± 9 years-old; $p < 0.001$) and female (53.7 vs. 32.6%; $p = 0.022$); they were also likely to smoke less (29.3 vs. 49.4%; $p = 0.031$), low HbA1c (7.1 vs. 6.3%; $p = 0.001$), and have a higher ARU (472 ± 70 vs. 431 ± 74; $p = 0.004$).

TABLE 3 | Factors associated with clopidogrel resistance after aspirin and clopidogrel treatment.

	Clopidogrel Resistance (–) (*n* = 89)	Clopidogrel Resistance (+) (*n* = 41)	*p*	OR*	95% CI	*P-value*
Age, years	68.7 (9.3)	75.0 (8.2)	<0.001	1.058	1.000–1.118	0.048
Male	60 (67.4)	19 (46.3)	0.022	0.406	0.170–0.970	0.042
Hypertension	71 (79.8)	31 (90.2)	0.139			
Diabetes mellitus	45 (50.6)	18 (43.9)	0.480			
Hyperlipidemia	70 (78.7)	33 (80.5)	0.810			
Smoking	44 (49.4)	12 (29.3)	0.031	-	-	-
History of CAD	29 (52.7)	11 (45.8)	0.573			
BMI (Kg/m^2)	24 (3.1)	24 (4.3)	0.432			
Laboratory findings						
C-reactive protein (mg/L)	1.2 (3.1)	2.4 (4.5)	0.121			
Hemoglobin A1c (%)	7.1 (1.7)	6.3 (1.0)	0.001	0.685	0.489–0.960	0.042
Aspirin reaction unit	431 (74)	472 (70)	0.004	1.007	1.001–1.013	0.018

Results are expressed by number (% column), mean (SD), or median (25–75 percentile range).
**Factors entered to model: age, sex, smoking, ARU, and CRP.*
CR, clopidogrel resistance; OR, odds ratio; CI, confidential interval; CAD, coronary artery disease; BMI, body mass index; NIHSS, National Institute of Stroke Scale; mRS, modified Rankin Scale.

From the results of the multivariate analysis, old age (OR = 1.058, 95% CI 1.000–1.118; $p = 0.048$), female sex (OR = 2.465, 95% CI 1.031–5.894; $p = 0.042$), low HbA1c (OR = 0.685, 96% CI 0.489–0.960; $p = 0.042$), and high ARU level (OR = 1.007, 95% CI 1.001–1.013; $p = 0.018$) were independently associated with clopidogrel resistance (**Table 3**). However, smoking was not significantly associated with clopidogrel resistance in the multivariate analysis.

Factors Associated With the PRU Level

The factors associated with the PRU level were age (beta = 0.014, 95% CI 0.004–0.025; $p = 0.010$) and ARU (beta = 0.002, 95% CI 0.001–0.004; $p = 0.001$; **Figure 2**), but not smoking. PRU increased with age in those receiving DAPT (Pearson $r = 0.235$, $p = 0.007$), but not in those receiving clopidogrel SAPT (Pearson $r = 0.033$, $p = 0.693$; **Figure 2**). Among those receiving DAPT, the ARU was significantly correlated with PRU (Pearson $r = 0.261$, $p = 0.003$) and percent inhibition (Pearson $r = -0.292$, $p = 0.001$; **Figure 2**).

DISCUSSION

In this study, HPR was more frequently observed in recurrent ischemic stroke patients receiving clopidogrel SAPT than in those receiving DAPT. Smoking was independently associated with low PRU and less clopidogrel resistance in recurrent ischemic stroke patients receiving clopidogrel SAPT, but not in those receiving DAPT. Instead, old age, female sex, low HbA1c, and high ARU were independent risk factors for clopidogrel resistance in recurrent ischemic stroke patients receiving DAPT. Age and the ARU level were associated with high PRU level.

Based on our result, HPR and biochemical clopidogrel resistance in smokers may help explain the mechanism underlying the smoker's paradox for clopidogrel. However, the smoker's paradox is still controversial. Smoking decreased short-term and in-hospital mortalities in several studies on CAD (14, 15). However, in long-term studies, a marked increase in long-term mortality negated the positive short-term outcomes (16). The *post-hoc* analysis of the CHANCE trial showed that the smoker's paradox may be observed in ischemic stroke patients receiving short-term DAPT (21 days) during the acute phase (6). However, whether the smoker's paradox will be observed after long-term DAPT in ischemic stroke patients is still unclear. Our results showed that smoking status did not determine HPR or clopidogrel resistance in recurrent ischemic stroke patients after long-term DAPT. Therefore, the smoker's paradox observed in acute ischemic stroke patients receiving short-term DAPT may differ from that observed in those receiving long-term DAPT.

According to treatment guidelines, DAPT is not routinely recommended for the secondary prevention of ischemic stroke (17). Therefore, most patients receive SAPT during the chronic stage of ischemic stroke (18). However, the guidelines for selecting the effective agent for SAPT for long-term secondary stroke prevention are insufficient. Our results showed that HPR was more observed in those with recurrent stroke receiving clopidogrel SAPT than those receiving DAPT. Therefore, when selecting an agent for long-term SAPT after DAPT, considering the factors affecting HPR may be important. In our study, more than 40% of the patients were still smoking at the time of stroke recurrence. In those receiving clopidogrel SAPT, the proportion of smokers was higher in those without than in those with clopidogrel resistance. Smoking was also an independent factor protective against clopidogrel resistance. Therefore, clopidogrel may be considered as a reasonable candidate for long-term secondary stroke prevention in those who fail to quit smoking.

Clopidogrel resistance was less observed in those receiving DAPT than in those receiving clopidogrel SAPT. Clopidogrel resistance may be a more important factor which determines the recurrence of stroke under the use of clopidogrel SAPT then DAPT. In the other hand, mechanisms other than HPR, such as a hemodynamic mechanism may have at least partially influenced the recurrence of stroke under DAPT. Factors associated with HPR also showed some differences between the two groups;

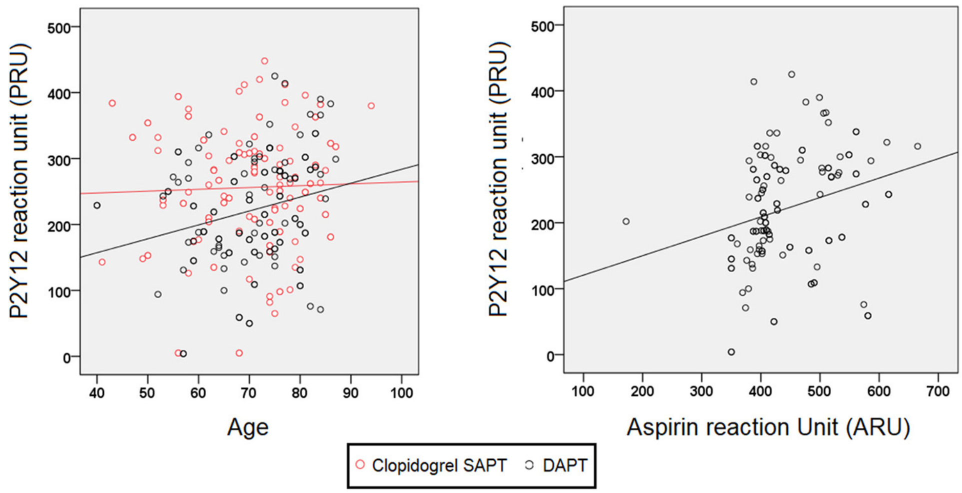

FIGURE 2 | PRUs stratified by age and ARU in recurrent stroke patients receiving DAPT.

hyperlipidemia and smoking status, which are well-known factors associated with HPR under clopidogrel, were associated with clopidogrel resistance in the SAPT group (19), whereas age, female, Hba1c level and ARU was associated with clopidogrel resistance in DAPT group. Age, and high ARU levels are also well-known risk factors of HPR under clopidogrel (20). As the resistance to aspirin was independently associated with clopidogrel resistance, a more common mechanism influencing HPR may have been involved in clopidogrel resistance in recurrent ischemic stroke patients under DAPT.

This study has several limitations. First, this study may have suffered from selection bias as it was retrospective. However, we have tried to minimize this by consecutively including recurrent ischemic stroke patients visiting to each center. For the same reason, we cannot have analyzed genetic testing (such as CYP2C19 loss of function). However, this study analyzed the retrospective data in "actual clinical practice." Tests related to CYP2C19 LOF alleles are generally not used in clinical practice. Second, it was impossible to analyze the timing of smoking cessation and the exact period of long-term DAPT before the recurrent stroke event due to the retrospective nature of this study. However, we attempted to determine smoking status by comparing past and recent records as soon as possible. Third, the information regarding previous strokes was more often diagnosed at different hospitals where it was first diagnosed. Therefore, accurate information about this was not available. Finally, we could not show the difference between stroke recurrence in smokers and non-smokers receiving long-term clopidogrel treatment. A well-designed study focusing on this may be needed.

We demonstrated that HPR is more frequent in recurrent stroke patients receiving clopidogrel SAPT than in those receiving DAPT. The rates of HPR and clopidogrel resistance were lower in current smokers. The authors believe that smoking is a major risk factor for ischemic stroke, and smoking cessation is necessary (21–24). However, we argue that it may be beneficial to consider the factors affecting HPR, such as smoking status, when selecting the SAPT agent for long-term secondary stroke prevention.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of Jeonbuk National University Hospital (approval number: CUH 2020-01-008). The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

HK and BK contributed to the design of this study and collected the raw clinical data. HK, SL, SH, D-iC, and BK contributed to the analysis of data, computational studies, and writing of the manuscript. HK contributed to this work as the first author. All authors read and approved the final manuscript.

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CASE REPORT
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Why Me? To Be an Ultra-Responder to Antiplatelet Therapy: A Case Report

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Background: Platelet function testing is a valid tool to investigate the clinical response to antiplatelet therapy in different clinical settings; in particular, it might supply helpful information in patients with cerebrovascular disease. Oral antiplatelet treatment, such as Aspirin (ASA) and Clopidogrel, is the gold standard in secondary stroke prevention of non-cardiogenic ischemic stroke; conversely, its application as a primary prevention therapy is not routinely recommended in patients with vascular risk factors. Multiple electrode platelet aggregometry (MEA) impedance aggregometer is a validated device to test platelet inhibition induced by ASA or Clopidogrel.

Case Report: We report the case of a 78-year-old patient without relevant clinical history, taking ASA as primary prevention strategy, who was admitted for sudden onset of dysarthria and left facial hyposthenia during physical effort. Brain CT revealed two small subcortical bilateral spontaneous intracranial hemorrhages. Platelet aggregometry with MEA performed upon admission revealed a very strong platelet inhibition induced by ASA (result of the ASPI Test was 5 U, consistent with an ultra-responsiveness to ASA, and the cutoff value of correct responsiveness is <40 U). MRI at longitudinal follow-up revealed the presence of two small cavernous angioma underlying hemorrhagic spots.

Conclusion: The evaluation of platelet reactivity in stroke patients undergoing antiplatelet therapies, not commonly performed in clinical practice, could be useful to optimize prevention strategies; the verification of the biological effectiveness of ASA or Clopidogrel could be a valid tool in the definition of each patient's risk profile, particularly in patients with cerebrovascular disease known to be at increased risk for both hemorrhagic and thrombotic complications.

Keywords: case report, aggregometry, antiplatelet therapy, primary prevention, intracerebral hemorrhage

BACKGROUND

International leading guidelines strongly recommended antiplatelet therapy in secondary prevention of non-cardiogenic strokes, as it is associated with an estimated reduction of relative risk of stroke or death on average by about 22% (1, 2). Conversely, the use of pharmacological strategy for primary cardiovascular prophylaxis, including stroke prevention, is still a debated topic (3). It is mandatory to improve the control of modifiable risk factors, such as hypertension and diabetes, but antiplatelet agents have no clear indications (3). Recently, the ACC/AHA guideline suggests to address primary prevention with low-dose Aspirin daily treatment to selected patients between 40 and 79 years of age, who are at higher risk for ischemic vascular event, but not at increased bleeding risk (4).

Therefore, the use of Aspirin might be reasonable only for people whose 10-year vascular risk is notable (at least higher than 10%) for the benefits to outweigh the risks associated with treatment. In particular, the association of diabetes mellitus with other high-risk conditions has been considered for primary prevention strategies (1, 3, 4).

Platelet function testing is a valid tool to investigate the clinical response to antiplatelet therapy in different clinical settings; several clinical and biological mechanisms for antiplatelet "resistance" or, conversely, "ultra-responsiveness" have been supposed (incongruent dose, poor compliance, genetic polymorphisms, baseline hyperactivity, and/or accelerated platelet turnover) (5–7). Thus, the possibility of testing the biological effectiveness of antiplatelet medications in vascular patients could be potentially useful for promptly detecting any relevant clinical problems, including safety in ultra-responder patients (8). However, the implementation of platelet function testing in routine clinical practice is not widely supported, mainly due to a lack of consensus on the effective improvement of clinical outcome with tailored therapy; other studies conversely debated the usefulness of platelet function monitoring, particularly in terms of reliability of results between different tests available (9, 10).

Within impedance aggregometers, the device "Multiple Electrode Platelet Aggregometry" (MEA, Multiplate Analyzer®, Roche Diagnostics International Ltd., CH-6343 Rotkreuz, Switzerland) (11, 12) showed correlation with the estimates of the antiplatelet effect of Clopidogrel and ASA obtained by other methods (13). Platelet aggregometry is a function test based on the stimulation of platelet–platelet aggregation with various agonists [adenosine diphosphate (ADP), arachidonic acid (ASPI), and thrombin receptor-activating peptide (TRAP)] and can be used to monitor the effects of antiplatelet agents, classified into three groups regarding their mechanism of action (thromboxane inhibitors—Aspirin, ASA, ADP receptor antagonists—Clopidogrel, and glycoprotein IIb/IIIa inhibitors). A comprehensive overview of platelet activation pathways is summarized in **Figure 1A**. According to the principles of impedance aggregometry, Multiplate Analyzer® assessed residual platelet function in whole blood of patients undergoing antiplatelet therapy; every test is performed in a single-use test cell, which incorporates two independent impedance metal sensors. After the addition of specific agonists (ADP, ASPI, and TRAP), the platelet–platelet aggregation is induced and real-time recording starts. The ADP Test reagent contains ADP, which triggers platelet activation *via* different ADP receptors, the most important of which is blocked by Clopidogrel (14). The ASPI Test reagent contains arachidonic acid, whose activation pathway is blocked by ASA (15); TRAP aggregation test is used to obtain a platelet aggregation measure relatively independent of others, supporting the proper sample preparation. Once activation of platelet aggregation starts on metal sensors, the electrical resistance increases; the resistance change is transformed to arbitrary aggregation units (AUs) and plotted against time. The area under the aggregation curve (AUC) quantifies the aggregation response, expressed in units (U; 1 U corresponds to 10 AU*min) (**Figure 1B**). Cutoff value of the ASPI Test indicating correct responsiveness to ASA is <40 U (16), while values under 30 U indicate strong enzymatic inhibition and higher risk of bleeding (17).

CASE PRESENTATION

A 78-year-old man without relevant clinical history was admitted in the Stroke Unit for sudden onset of slurred speech and left oral rhyme deviation during physical effort, without headache and/or limb weakness. Patient's past medical history reported bilateral neurosensory hypoacusis, previous cataract surgery, and carpal tunnel syndrome surgically treated. Pharmacological anamnesis revealed daily treatment with Aspirin 100 mg as a vascular primary prevention strategy, started 3 months before.

Neurological examination showed paralysis of right VII cranial nerve, right deviation of protruded tongue, and mild dysarthria (NIH stroke scale 2/42). Brain CT revealed multiple chronic lacunar infarctions of basal ganglia bilaterally, and two acute small intraparenchymal hemorrhages, within post-rolandic subcortical region on the right side (**Figure 2A**) and pre-rolandic subcortical region on the left side (**Figure 2B**); CT angiography showed mild carotid and vertebral atherosclerosis, and no vascular malformation (**Figure 2C**).

Aspirin therapy was immediately discontinued. Intensive monitoring in the Stroke Unit and cardiac ultrasound revealed an unknown arterial hypertension, with a chronic hypertensive cardiopathy. Target therapy with ACE inhibitors (Enalapril 20 mg once daily) was started, with blood pressure normalization. Multiplate® platelet function analysis performed upon admission revealed a very strong platelet inhibition induced by ASA; the area under the aggregation curve (AUC) on the ASPI Test was 5 U, consistent with an ultra-responsiveness to ASA, with normal platelet aggregation induced by other agonists on the ADP Test and TRAP Test (**Figure 3**).

Neurological examination of patients at discharge was completely normalized. Due to the "atypical" locations of intraparenchymal hematomas, we performed a brain MRI at longitudinal follow-up in order to exclude non-hypertensive causes of bleeding. Gradient-echo T2*-weighted sequences revealed two small roundish lesions, in the anatomical site of bilateral subcortical hematomas, with minute central

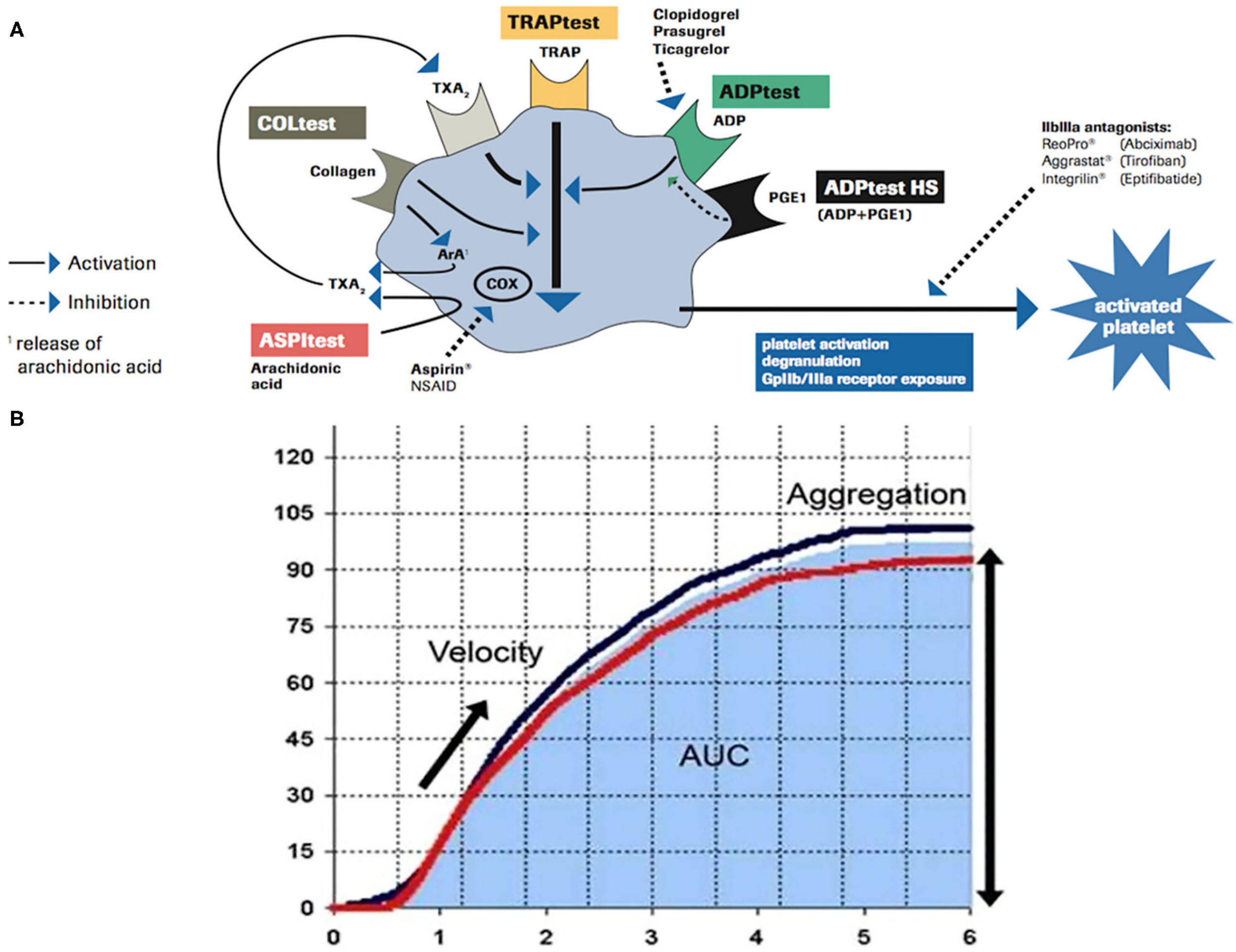

FIGURE 1 | (A) Schematic overview of platelet activation/inhibition pathways and impedance aggregometry tests (ADP Test, ASPI Test, and TRAP Test). **(B)** Graphic presentation of platelet–platelet aggregation induced during each test; platelet responsiveness is quantified by the area under the curve (AUC*min). Modified from Roche Diagnostics International.

nucleus of methemoglobin and dark hemosiderin rim, and without surrounding edema, consistent with cavernous venous malformations (**Figure 4A**). Multiple similar but smaller cavernous angiomas were detected throughout subcortical white matter on both sides, particularly in temporal and occipital lobes, and in the area of basal ganglia (**Figures 4B,C**). On differential diagnosis, T2 and FLAIR sequences excluded findings suggestive of other conditions, as possible cerebral amyloid angiopathy; no evidence of significant subcortical leukoencephalopathy was detected besides lacunar microinfarcts in the region of basal ganglia bilaterally, and no signs consistent with superficial siderosis were detected.

DISCUSSION

We presented a case of a previous healthy patient, admitted for intracerebral atypical hemorrhages, taking no medications except ASA in primary prevention. Diagnostic workup revealed a condition of unrecognized arterial hypertension, and the presence of multiple intracerebral cavernous venous malformations, some of which with acute bleeding. Symptomatic hemorrhagic complication occurs as a clinical manifestation of cavernous angioma in 25% of cases (18), but the annual average rate of bleeding is reported to be lower in patients without history of prior hemorrhage (19). However, rupture rate rises in patients with associated condition at risk of bleeding, such as hypertension. Many studies suggest the likely safety of antiplatelet medications in patients with cerebral cavernous malformations (19), but outside of randomized controlled protocols (20).

The role of antiplatelet agents for the primary prevention of cardiovascular disease, including stroke, is still widely debated, due to the delicate balance between efficacy and safety in patients without established previous vascular events. Several

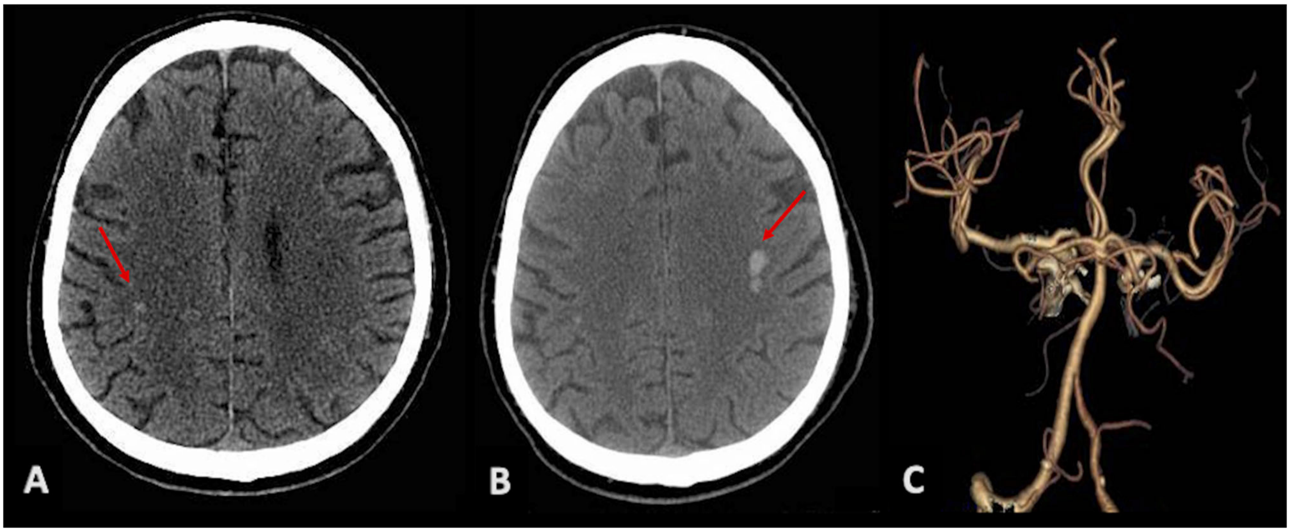

FIGURE 2 | Brain CT scans showing bilateral intraparenchymal hyperdense lesions (red arrows): a small hemorrhagic spot in the post-rolandic area on the right **(A)** and a greater hematoma in the left pre-rolandic area **(B)**. **(C)** CTA with no evidence of vascular malformations.

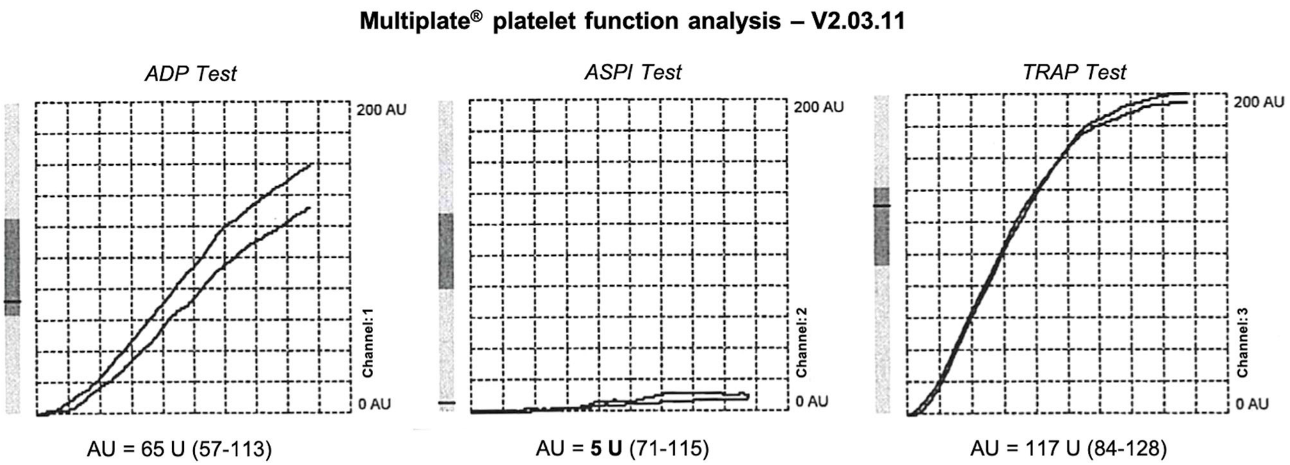

FIGURE 3 | Platelet function testing performed upon admission during Aspirin therapy. Marked reduction of AUC on ASPI Test, 5 U, expressing a strong platelet inhibition induced by ASA (ultra-responder patient). Expected values in healthy individuals are in brackets.

randomized clinical trials showed that Aspirin is effective in the reduction of recurrence risk, with a tolerable increase of bleeding complications; thus, international practice guidelines strongly recommended ASA in secondary prevention of vascular diseases, as ischemic stroke or myocardial infarction (1). Regarding primary prevention, diverging results have contributed to unclear indications about antiplatelet therapy, which is not routinely recommended, primarily due to safety (4). Therefore, in clinical practice, ASA treatment should be tailored on each patient's risk profile (e.g., associations of diabetes mellitus and other high-risk conditions) and might be reasonable only in case of a notable 10-year risk of primary vascular events (3, 4).

The possibility to test the biological effectiveness of antiplatelet agents, with platelet function testing devices such as Multiplate Analyzer®, might supply helpful information to clinicians, primarily to assess the responsiveness to ASA or Clopidogrel in ischemic stroke patients. Nevertheless, it might be a valid tool in the stratification of patient's risk profile as well, while considering the safety of a primary prevention regimen, particularly in the presence of clinical conditions associated with an increased risk of hemorrhagic complications.

However, longitudinal studies are needed to assess whether aggregometry might supply individualized information and whether it can be considered a valid

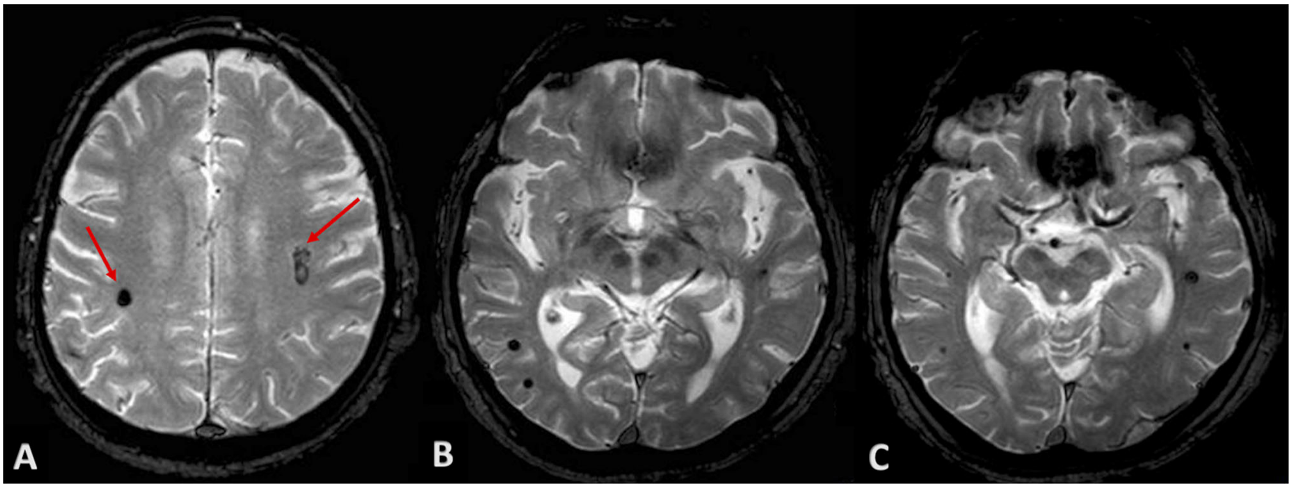

FIGURE 4 | Patient's MRI at follow-up. Gradient-echo T2*-weighted sequences revealed roundish lesions underlying well-known intraparenchymal hemorrhages (**A**, red arrows), with classic magnetic resonance appearance of cavernous venous malformations. **(B,C)** show multiple and similar lesions throughout subcortical white matter and basal ganglia, bilaterally.

tool in the development of tailored therapies, as the main limitation of its implementation in everyday clinical practice.

CONCLUSION

Our report illustrates the potential clinical benefit of platelet function testing in patients undergoing antiplatelet therapy, with particularly useful application in the definition of patient's risk profile in case of primary prevention treatment with Aspirin. However, RCTs and longitudinal studies are needed to assess whether routine platelet function monitoring might be considered a decision-making tool for clinicians, both in patients with vascular diseases subjected to secondary prevention therapy and during the evaluation of safety profile of antiplatelet treatment in selected patients deserving of pharmacological primary prevention therapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FR, GB, and AZ: manuscript drafting/revising, data acquisition, and final revision. NL: data acquisition. LV: manuscript revising and data acquisition. FN: manuscript revising. LC: manuscript revising and data acquisition. SM: manuscript drafting/revising and final revision. All authors approved the final version.

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Effects of Prior Antiplatelet Therapy on Mortality, Functional Outcome, and Hematoma Expansion in Intracerebral Hemorrhage: An Updated Systematic Review and Meta-Analysis of Cohort Studies

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Background and Objective: Antiplatelet therapy (APT) is widely used and believed to be associated with increased poor prognosis by promoting bleeding in patients with intracerebral hemorrhage (ICH). We performed a systematic review and meta-analysis to determine whether prior APT is associated with mortality, functional outcome, and hematoma expansion in ICH patients.

Methods: The PubMed, Embase, and Web of Science databases were searched for relevant published studies up to December 11, 2020. Univariate and multivariable adjusted odds ratios (ORs) were pooled using a random effects model. Cochran's chi-squared test (Cochran's Q), the I^2 statistic, and meta-regression analysis were used to evaluate the heterogeneity. Meta-regression models were developed to explore sources of heterogeneity. Funnel plots were used to detect publication bias. A trim-and-fill method was performed to identify possible asymmetry and assess the robustness of the conclusions.

Results: Thirty-one studies fulfilled the inclusion criteria and exhibited a moderate risk of bias. Prior APT users with intracerebral hemorrhage (ICH) had a slightly increased mortality in both univariate analyses [odds ratio (OR) 1.39, 95% CI 1.24–1.56] and multivariable adjusted analyses (OR 1.41, 95% CI 1.21–1.64). The meta-regression indicated that for each additional day of assessment time, the adjusted OR for the mortality of APT patients decreased by 0.0089 (95% CI: −0.0164 to −0.0015; P = 0.0192) compared to that of non-APT patients. However, prior APT had no effects on poor function outcome (pooled univariate OR: 0.99, 95% CI 0.59–1.66; pooled multivariable adjusted OR: 0.93, 95% CI 0.87–1.07) or hematoma growth (pooled univariate OR: 1.23, 95% CI 0.40–3.74, pooled multivariable adjusted OR: 0.94, 95% CI 0.24–3.60).

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Conclusions: Prior APT was not associated with hematoma expansion or functional outcomes, but there was modestly increased mortality in prior APT patients. Higher mortality of prior APT patients was related to the strong influence of prior APT use on early mortality.

Systematic Review Registration: PROSPERO identifier [CRD42020215243].

Keywords: antiplatelet therapy, intracerebral hemorrhage, mortality, functional outcome, hematoma expansion

INTRODUCTION

Spontaneous intracerebral hemorrhage (ICH) in patients taking antiplatelet therapy (APT) is common in routine clinical practice (1, 2). Antiplatelet therapy (APT) has attracted wide attention because of its beneficial effects on cardiovascular and cerebrovascular diseases (3). Approximately 20% to 30% of patients with ICH are on APT (4, 5). However, prior APT is believed to be associated with increased mortality and poor prognosis due to the promotion of bleeding in patients with ICH (6). Several studies have reported that ongoing hemorrhage expansion is an independent predictor of increased mortality and poor functional outcome following ICH (7).

Previous studies regarding the prognosis of prior APT in patients with ICH have shown conflicting results. Some suggest that an increased risk of death and poor outcome are associated with prior APT (5, 8, 9), while others suggest the opposite association (10–13). A meta-analysis published in 2010 (4) found higher mortality in ICH patients with prior APT. Recently, several large cohort studies reported that prior APT was not associated with significant death and disability (10, 12, 13). Overall, whether prior APT is associated with higher mortality, poor outcome, or hematoma expansion in ICH patients remains unclear. Given the conflicting data between APT and ICH outcomes, the current American Heart Association and European Stroke Organization guidelines for the routine use of platelet transfusion after ICH are inconclusive (14). Meanwhile, it is not clear whether APT use is related to hematoma enlargement. Thus, it is worthwhile to perform an updated systematic review and meta-analysis to determine the correlation between prior APT use and ICH outcomes.

METHODS

Search Strategy

This meta-analysis was registered in the International Prospective Register of Systematic Reviews (PROSPERO; Registration NO. CRD42020215243) and conducted following the guidelines of the Cochrane Handbook for Systematic Reviews of Intervention and the PRISMA statements (15). Two authors systematically searched the following databases from inception to December 11, 2020: PubMed, Embase, and Web of Science. The following terms were used to identify eligible studies: ("ICH" OR "intracerebral hemorrhage" OR "intracerebral") AND ("APT" OR "antiplatelet"). No language restriction was applied. In addition, we also performed a manual search of the references in relevant articles to retrieve eligible studies.

Inclusion Criteria

The inclusion criteria were as follows: (1) cohort studies included consecutive patients with the primary outcome or the secondary outcome of ICH; (2) ICH patients were all verified by computed tomography or magnetic resonance imaging; (3) prior APT was one of the influencing analysis factors; (4) the adjusted or unadjusted odds ratio (OR) with aspect among mortality, poor function outcome, and hematoma growth between ICH patients with and without prior APT could be acquired directly or by calculation; and (5) primary outcome: mortality after intracranial hemorrhage in consecutive patients; secondary outcomes: (a) poor function outcome, defined as being within a specific scoring range using widely accepted validated scales [the modified Rankin Scale (mRS) or the Glasgow Scale Score (GSS)]; (b) hematoma growth was defined as an increase in the baseline hematoma volume by either 33% or >6 ml on the interval CT scan performed within 72 h.

Exclusion Criteria

(1) Patients included secondary cerebral hemorrhage caused by trauma, tumor, aneurysm rupture, or arteriovenous malformation. (2) Studies cannot strictly separate ICH patients with APT and without APT due to a lack of detailed information. (3) Studies without enough information to judge the effectiveness of the statistical methods.

Study Selection

Two authors (YW and DZ) independently reviewed the identified studies. Full texts of potentially relevant articles were retrieved after screening titles and abstracts. Any disagreement was discussed with the third author (HC).

Data Extraction

Two authors (YW and DZ) independently extracted the following data from eligible studies: study characteristics (first author, year of publication), study range (single-center or multicenter), study type (prospective or retrospective), study continent, study reputation (the mean-centered impact score of the journal the study was published in), patient age, and assessment time. Since the combination of APT and other anticoagulant drugs would lead to a change in the drug mechanism, we extracted the data of the patients who take APT alone in this paper. Meanwhile, we extracted data on mortality, poor function outcome, and hematoma growth at all time points in all included studies. Mortality data were divided into early time, 30-, 90-day, and discharge groups to compare the differences between groups.

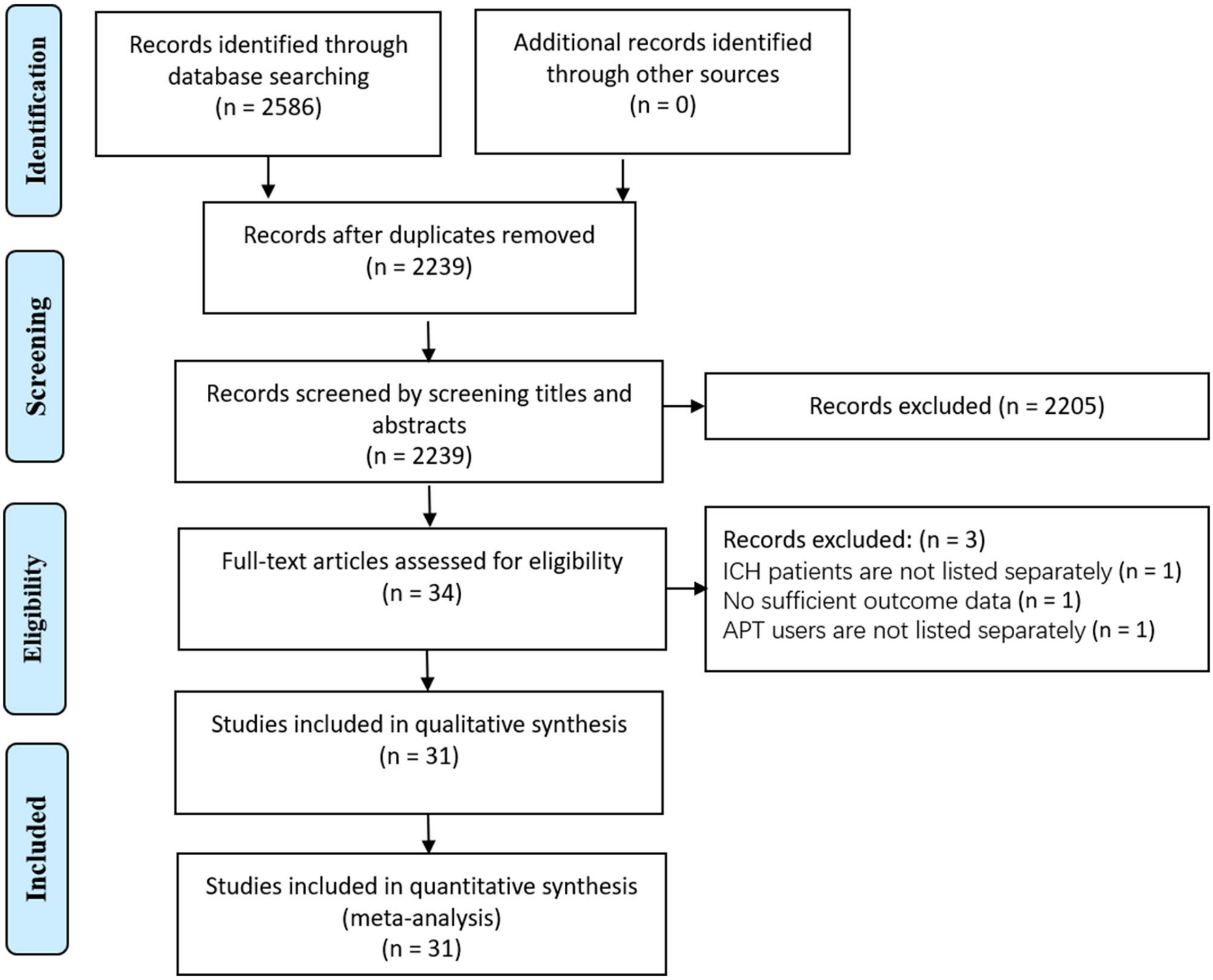

FIGURE 1 | Study flowchart.

Risk of Bias Assessment

Risk of bias was assessed by two investigators (YW and DZ) with the Robins-I tool for non-randomized studies. The following domains for the non-randomized studies were evaluated: confounding, selection of participants, departure from intended interventions, missing data, measurement of outcomes, and selective reporting at low, moderate, serious, or critical risk. These domains were combined to result in an overall risk of bias judgment as low, moderate, serious, or critical. Discrepancies in risk of bias assessment were resolved *via* discussion (16).

Statistical Analysis

This meta-analysis was performed using the R software (version 4.0.2, 64 bits, The Cochrane Collaboration, Oxford, UK). Raw data containing valid results were calculated as odds ratios for statistical analysis. To ensure the reliability of the study, we separately pooled the adjusted OR or unadjusted OR with a 95% confidence interval (95% CI) as the effect size of this meta-analysis. Cochran's chi-squared test (Cochran's Q) and I^2-test were used to analyze heterogeneity among the studies. According to the Cochrane Review guidelines, the threshold for heterogeneity is an $I^2 < 50\%$ and a $P < 0.1$ and indicated using a random effects model in OR computation rather than a fixed effects model (17). Furthermore, based on a literature review and clinical experience, the possible variables that may cause heterogeneity, including publication year, study center, study type, study continent, and study reputation, were analyzed by univariate meta-regression. $P < 0.05$ was considered the cause of heterogeneity. On the other hand, we performed subgroup analysis based on the assessment time. In addition, we separately performed a meta-regression analysis to explore the relationship between assessment time and mortality under prior APT use. Sensitivity analysis was then carried out by excluding each study one by one. Publication bias was assessed both visually evaluating the symmetry of the funnel plot and mathematically using the Egger regression intercept for outcomes. P-values < 0.05 were identified as significant publication bias (18).

RESULTS

Results of the Literature Search

The search of electronic bibliographic sources retrieved a total of 2,586 studies. After screening the title, abstract, and full text, 31 cohort studies met our eligibility criteria and were included in the analysis. The PRISMA flow diagram for the selection is presented in **Figure 1**.

Study Characteristics

All 31 studies (5, 6, 8–13, 19–41) had an observational design, of which 17 studies (6, 8–10, 12, 13, 20, 25, 26, 28–30, 32, 35, 38, 40, 41) were retrospective relying on medical records, and the other 14 studies (5, 11, 19, 21–24, 27, 31, 33, 34, 36, 37, 39) were prospective cohort studies. Twenty studies (6, 10, 11, 13, 20, 22, 23, 25–31, 33, 35, 36, 38, 39, 41) were single-center studies, while the rest were conducted in more than one institution. Among the studies, 14 were conducted in Europe (5, 10, 11, 13, 21, 23–25, 27–29, 35, 39, 40), 6 were conducted in America (12, 22, 30, 31, 33, 39), and 11 were conducted in Asia (6, 9, 19, 20, 26, 32, 34, 36–38, 41). In total, 219,726 patients were included, of which 50,285 underwent APT therapy (weighted mean proportion 22.9%, range 4.3–44.9%). Generally, patients on APT were older and had more cerebrovascular risk factors across most studies (5, 9–13, 24–36, 38, 39). The characteristics of the included studies are summarized in **Table 1**.

Risk of Bias in Included Studies

All included studies were rated as having a moderate or serious risk of bias with the ROBINS-I tool (**Figures 2A,B**). Twenty-three studies (6, 8–13, 22–33, 36, 38, 39, 41) had a moderate risk of bias since they were generally involved with adjustment for confounding, although possible residual confounding could not be excluded. The other seven studies (5, 19–21, 34, 35, 40) were judged as having a serious risk of bias, mainly due to a lack of control for confounding and measurement bias in studies relying on retrospective medical records.

Results of Meta-Analysis

Primary Outcome

Effects of Prior APT on Mortality of ICH Patients

The mortality of ICH patients was reported in 28 studies (5, 6, 8–13, 19, 21–35, 37–40) with 218,530 patients. Five studies (10, 28, 29, 33, 39) reported mortality at more than one time point. A total of 26 cohorts (210,842 patients) (6, 8–11, 13, 19, 21–35, 37–40) contributed data for the univariate mortality analysis, and 15 cohorts (203,969 patients) (5, 8, 9, 12, 13, 19, 23–26, 28, 30, 32, 37, 39) provided data for the multivariable adjusted mortality analysis. From both pooled univariate ORs and pooled multivariable adjusted ORs, we found that prior APT was significantly associated with higher mortality (OR 1.39, 95% CI 1.24–1.56; OR 1.41, 95% CI 1.21–1.64). However, substantial heterogeneity was detected for both univariate analyses and multivariable analyses ($I^2 = 83\%$, $P < 0.001$; $I^2 = 70\%$, $P < 0.001$) (**Figures 3A,B**).

To determine the source of heterogeneity, meta-regression analyses were conducted, and the results are presented in **Tables 2**, **3**. The results revealed that the effect size was significantly correlated with different study centers, study types, and continents ($p < 0.05$) in univariate analyses and multivariable analyses.

Subgroup Analysis and Meta-Regression

To test the hypothesis that the assessment time could be an essential factor in mortality between APT and non-APT patients, we performed a series of subgroup analyses and meta-regression analyses based on the time of assessment.

In the subgroup analysis, we divided the included studies into four groups: early time (1–14 days), 30 days (21–30 days), 90 days, and discharge according to the evaluation time included. As shown in **Figures 4A,B**, the pooled unadjusted ORs for mortality were 1.49 (95% CI: 0.83–2.96), 1.28 (95% CI: 0.91; 1.80), 1.82 (95% CI: 1.38; 2.41), and 1.26 (95% CI: 1.08; 1.48) for each group, respectively. Similarly, the pooled adjusted OR for mortality of each group was 2.85 (95% CI: 1.59–5.09), 3.03 (95% CI: 1.96–4.69), 1.59 (95% CI: 1.07–2.35), and 1.11 (95% CI: 1.01–1.22), indicating that the relationship between prior APT use and mortality varied in different time periods.

To further explore the relationship between death events and assessment time, we conducted a meta-regression based on assessment time, excluding the time point at discharge for its variability. We found no significant association between the unadjusted OR for mortality and assessment time ($P = 0.4216$; **Figure 4C**). However, there was a significant trend regarding multivariable adjusted analyses, with the adjusted OR for mortality of APT patients decreasing by 0.0089 for each additional day of assessment time (95% CI: −0.0164 to −0.0015; $P = 0.0192$) (**Figure 4D**) compared to non-users.

Secondary Outcomes

Effects of Prior APT on the Outcome in ICH Patients

Ten studies (11, 20, 27, 30, 32–35, 38, 41) with a total of 3,622 patients under univariate analyses and five studies (8, 12, 24, 31, 39) with a total of 86,201 patients under multivariable adjusted analyses reported the effects of prior APT on the poor functional outcome of ICH patients. Studies that did not report scale results were not included for poor prognosis analysis. No significant difference was found in the poor functional outcome between prior APT patients and no prior APT patients regarding either pooled unadjusted ORs or multivariable adjusted ORs (OR 0.99, 95% CI 0.59–1.66; OR 0.93, 95% CI 0.87–1.07) (**Figures 5A,B**). The between-study statistical heterogeneity was substantial for univariate analyses ($I^2 = 82\%$, $P < 0.001$).

Effects of Prior APT on Hematoma Growth in ICH Patients

Four studies with a total of 1,052 patients (6, 31, 37, 41) reported the effects of prior APT on HG with univariate ORs, and seven studies (6, 11–13, 32, 35, 36) reported this outcome with multivariable adjusted ORs (including 3,518 patients). The incidence of hematoma expansion was

TABLE 1 | Characteristics of eligible studies.

References	Study type	Continent	Study population	No.	Mean age (SD)	Apt mean age (SD)	Not apt mean age (SD)	Male %	Pre-ICH APT, %	Time of assessment	All mortality (%)	Definition of poor outcome
Camps-Renom et al. (11)	Prospectively	Europe	Single center	223	72.5 (13)	77.3 (10)	70.1 (3.7)	54.3	74 (33.2)	90 days	31.4	mRS 3-6
Gulati et al. (40)	Retrospectively	Europe	Multicenter	19,921	NR	66.1	NR	NR	5,865 (29.4)	90 days	25.1	mRS 3-6
Hallevy et al. (20)	Retrospectively	Asia	Single center	169	71.2	NR	NR	54.4	18.5	Discharge	33	mRS 4–6
Nilsson et al. (21)	Prospectively	Europe	Multicenter	338	74	NR	NR	56	74 (21.9)	30 days	36	NR
Rosand et al. (22)	Prospectively	America	Single center	435	74.4 (9.3)	NR	NR	48.2	139 (32.0)	90 days	32	NR
Toyoda et al. (6)	Retrospectively	Asia	Single center	251	66	NR	NR	60.6	57(42.1)	Discharge	12.4	NR
Roquer et al. (23)	Prospectively	Europe	Single center	387	71.6 (12.5)	NR	NR	55.2	47 (24.2)	30 days	26.3	NR
Foerch et al. (24)	Prospectively	Europe	Multicenter	1,483	72 (12)	75 (10)	70 (14)	52	441 (26)	Discharge	22.7	mRS 3-6
Karlikaya et al. (25)	Retrospectively	Europe	Single center	664	NR	67.1 (12.5)	65.8 (13.3)	NR	40 (6.0)	21 days	28	mRS 3-6
Saloheimo et al. (26)	Retrospectively	Asia	Single center	182	NR	71.6 (11.2)	65.6 (11.1)	49.5	44 (24.1)	90 days	32.7	NR
Caso et al. (27)	Prospectively	Europe	Single center	457	NR	78.9 (9.0)	73.8 (9.4)	58	94 (20.5)	Discharge	23.2	GOS 1–3
Lacut et al. (28)	Retrospectively	Europe	Single center	138	NR	70.5	61.5	60.9	30 (21.7)	7/30/90 days	20.29	mRS 4–6
Hanger et al. (29)	Retrospectively	Europe	Single center	223	NR	75.7	69.9	48.9	91(39.2)	8/14/28days	42.3	mRS 3-6
Creutzfeldt et al. (30)	Retrospectively	America	Single center	368	72.5	70 (12)	62 (17)	50.3	121 (31.3)	Discharge	34.5	mRS 3-6
Sansing et al. (31)	Prospectively	America	Single center	282	NR	71	63	66	70 (24.8)	90 days	17.2	mRS 3-6
Toyoda et al. (32)	Retrospectively	Asia	Multicenter	918	NR	71 (10)	65 (13)	59.3	180 (19.6)	21 days	13.2	mRS 3-6
Stead et al. (33)	Prospectively	America	Single center	178	NR	79	66	49.4	80 (44.9)	7/30 days	27	mRS 2-6
Balci et al. (34)	Prospectively	Asia	Multicenter	337	NR	70.1 (10.9)	67.2 (11.2)	44.5	48 (14.2)	Discharge	36.5	mRS 3-6
Kuramatsu et al. (35)	Retrospectively	Europe	single-center	210	69.6 (11.7)	72.2 (11.0)	67.9 (11.9)	51.9	83 (39.5)	90 days	31.4	mRS 4–6
Yildiz et al. (36)	Prospectively	Asia	Single center	153	66 (12)	70 (11)	64 (12)	61.4	52 (34)	NR	NR	mRS 3-6
Chen et al. (10)	Retrospectively	Europe	Single center	1,927	NR	68.4 (11.7)	61.5 (14.5)	63.7	232 (12)	30 days	15	NR
Mansouri et al. (37)	Prospectively	Asia	Multicenter	90	64.6	NR	NR	NR	26 (28.9)	90 days	47	NR
Yang et al. (38)	Retrospectively	Asia	Single center	333	NR	65.4(12.6)	57.5(13.9)	40	68(20.4)	Discharge	46.7	GCS worsen
Stein et al. (5)	Prospectively	Europe	Multicenter	7,051	NR	77.2 (10.0)	70.1 (14.1)	48	2,113 (30.0)	Discharge	23.2	NR
Roquer et al. (39)	Prospectively	Europe	Single center	440	NR	80	74	50.9	147(33.4)	1/90 days	NR	NR
Khan et al. (8)	Retrospectively	America	Multicenter	82,576	64	NR	NR	NR	28,277 (34.2)	Discharge	24.2	mRS 3-6
Hokari et al. (41)	Retrospectively	Asia	Single center	429	NR	NR	NR	58.3	64 (14.9)	30 days	NR	NR
van Ginneken et al. (13)	Retrospectively	Europe	Single center	343	NR	77	72	49.3	99 (29)	Discharge	10	mRS 5-6
Liu et al. (9)	Retrospectively	Asia	Multicenter	97,355	NR	69	NR	64.4	11,351 (11.7)	Discharge	NR	NR
Murthy et al. (12)	Retrospectively	America	Multicenter	1,420	NR	66.5 (11.6)	61.3 (12.5)	NR	284 (20)	Discharge	NR	mRS 4–6
Wong et al. (19)	Prospectively	Asia	Multicenter	783	61.3 (15.02)	NR	NR	69	34 (4.3)	Discharge	29.8	mRS 4–6

NR, not reported; APT, antiplatelet therapy; ICH, intracerebral hemorrhage; mRS, modified Rankin Scale; GSS, Glasgow Scale Score.

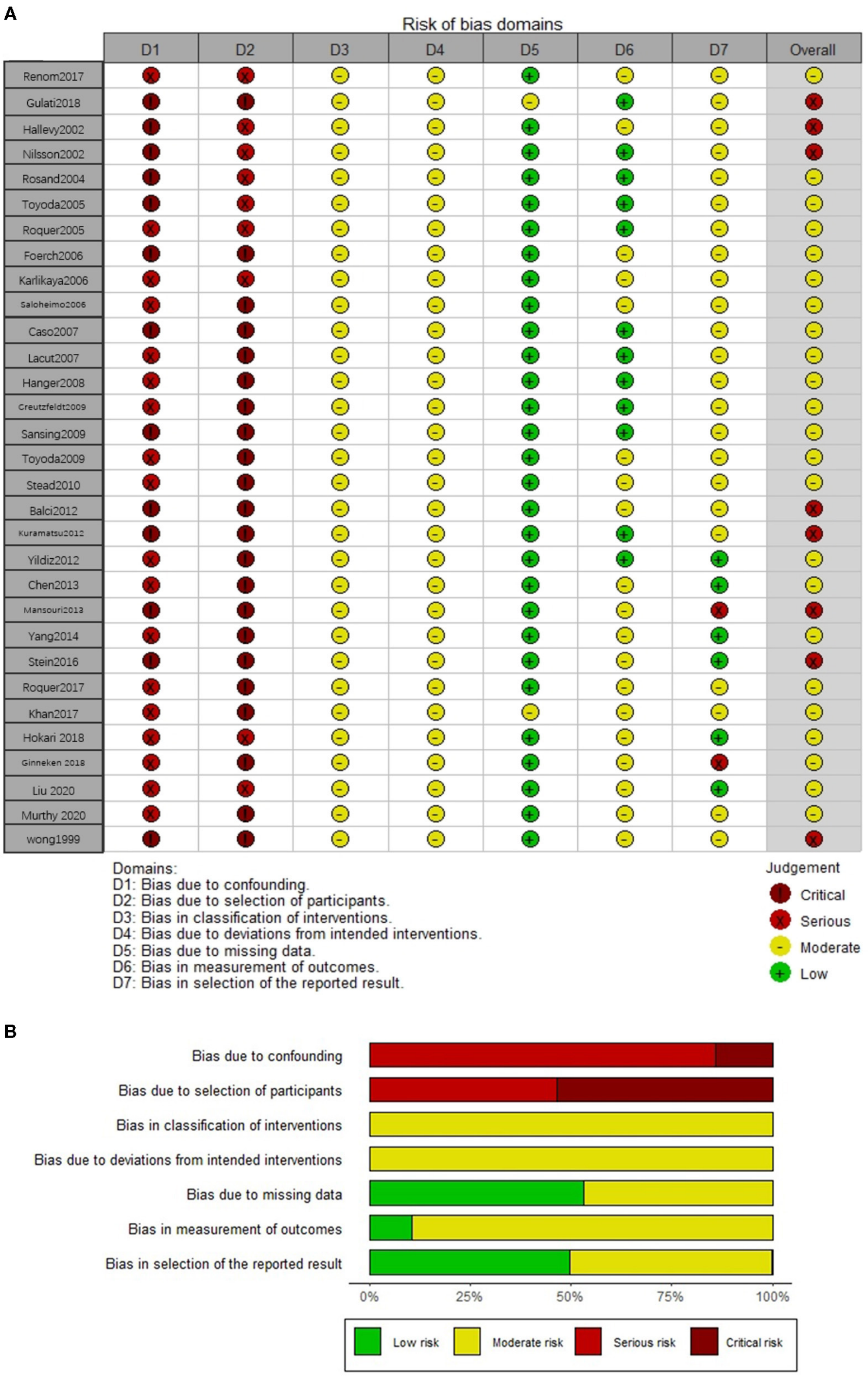

FIGURE 2 | The risk of bias assessment of each included study **(A)** and weighted summary of the risk of bias **(B)**.

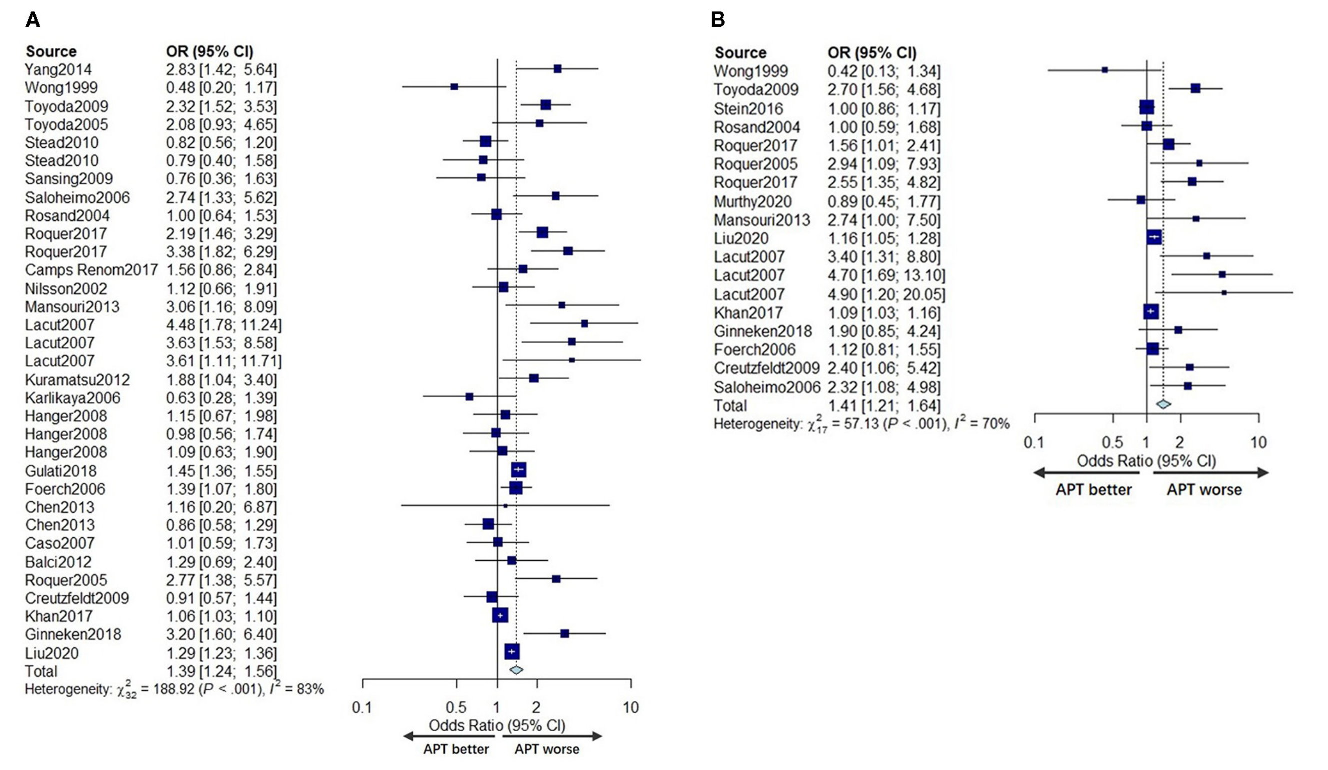

FIGURE 3 | Forest plot comparing mortality for APT vs. no APT for univariate analyses **(A)** and multivariable adjusted analyses **(B)**.

TABLE 2 | Meta-regression results of univariate analyses.

Variables		Regression coefficient (SE)	95% CI	*p*-value
Published year		0.011	–0.0062–0.0369	0.1619
Population	Single center	0.0765	0.2292–0.5291	<0.0001*
	Multicenter	0.0901	0.0777–0.4308	0.0048*
Study type	Prospective	0.0932	0.0873–0.4526	0.0038*
	Retrospective	0.0758	0.2175–0.5145	<.0001*
Reputation		0.0443	–0.0353–0.1383	0.2451
Continent	America	0.1396	–0.3574–0.1897	0.5481
	Asia	0.1408	0.1247–0.6767	0.0044*
	Europe	0.0896	0.3175–0.6688	<0.0001*

*$p < 0.05$.

not significantly different between prior APT users and non-users in either univariate analyses or multivariable adjusted analyses (OR 1.23, 95% CI 0.40–3.74, OR 0.94, 95% CI 0.24–3.60) (**Figures 5C,D**). The statistical heterogeneity was also moderate ($I^2 = 66\%$, $P = 0.03$, $I^2 = 64\%$, $P = 0.01$).

Publication Bias and Sensitivity Analysis

Funnel plots and Egger's-test were used to reveal possible publication bias. The results showed no overestimation of effect sizes except for the analysis of mortality in studies with univariate ORs and multivariable adjusted ORs (Egger's test: $P = 0.4$, $P = 0.002$) (**Table 4**). Next, the trim-and-fill method was applied to evaluate the impact of publication bias on our meta-analysis results. After seven studies and six studies were separately filled and no studies trimmed, the OR was not significantly changed (OR = 1.22, 95% CI 1.09–1.37, OR =1.21, 95% CI 1.02–1.42) (**Figures 6G,H**), suggesting that publication bias had little effect on the results. Funnel plots are shown in **Figure 6**.

TABLE 3 | Meta-regression results of multivariable adjusted analyses.

Variables		Regression coefficient (SE)	95% CI	*p*-value
Published year		0.0138	−0.0456–0.0084	0.1777
Population	Single center	0.1204	0.4158–0.8878	<0.0001*
	Multicenter	0.0763	−0.0174–0.2817	0.0832
Study type	Prospective	0.1247	0.0118–0.5007	0.0399*
	Retrospective	0.1158	0.2352–0.6891	<0.0001*
Reputation		0.0534	−0.1620–0.0472	0.2821
Continent	America	0.2431	−0.3149–0.6381	0.5063
	Asia	0.2624	−0.1222–0.9065	0.1351
	Europe	0.1702	0.3138–0.9810	0.0001*

*$p < 0.05$.

DISCUSSION

Summary of Main Results

The present meta-analysis was conducted to explore the effects of prior APT on mortality, functional outcome, and hematoma growth in patients with ICH. The meta-analysis demonstrated that prior APT users had a slightly increased mortality. However, prior APT had no effects on the poor functional outcome or hematoma growth in patients with ICH.

Primary Outcome

Clarifying the relationship between prior APT and ICH mortality is important because a large portion of the general population regularly takes these drugs, and their usage is likely to increase as the population ages (42, 43). Furthermore, restoration of normal platelet function could be a therapeutic target if prior APT worsens the outcome of ICH patients. This present meta-analysis showed that prior APT led to increased mortality, as reported by a previous study (4). The determination of mortality has high between-group reliability and is less susceptible to the determination bias associated with the study design. Moreover, the pooled results in this meta-analysis were consistent with both unadjusted ORs and adjusted ORs. Although it is difficult to completely eliminate the influence of all confounding factors, the above factors indicate the high reliability of our results.

The included studies in previous reviews (4) were published from 1998 to 2010, and the majority of them were studies published around 2005, which is a long time ago. Fifty-three percent of the studies (5, 8–13, 34–41) included in our present meta-analysis were published after 2010, which reported conflicting results. Therefore, an updated meta-analysis is needed. Additionally, we collected data with more assessment time points, such as early access at 30 days before, and conducted a meta-regression analysis based on assessment time to further explore whether the relationship between prior APT use and mortality changed over time of assessment. Based on the above analysis, we also analyzed the relationship between APT and hematoma dilatation.

In the subgroup analysis, the results indicated that early-time death was more frequent in patients on prior APT in multivariable adjusted analyses, the same as that at 90 days. However, at 30 days, the relationship became insignificant. These results were similar to those reported by Roquer's prospective study (39). Roquer believed that the higher mortality of prior APT patients was related to the strong influence of APT pretreatment on early mortality, and 90-day mortality seems to be a subrogation of early-time mortality. Therefore, to verify the above hypothesis, we performed a further meta-regression analysis to explore the linear relationship between assessment time and the effect of prior APT use on mortality and found consistent results. We found that the effect of prior APT on mortality use decreased over time in multivariable adjusted analyses. Since the platelet life was 7–10 days, with an ∼10% rate of daily updates (44), prior APT patients who present with higher early-time mortality are believed to have insufficient platelet activity early in life (6, 26, 32). However, in univariate analyses, death was significantly more frequent in patients on prior APT only in the 90-day group, and no significant association was found. This difference might be explained by the fact that patients pretreated with APT were older and had poor previous functional status and more vascular risk factors than the non-pretreated group (5, 9–13, 24–36, 38, 39). In addition, long-term discontinuation of APT may worsen cardiovascular and cerebrovascular conditions and lead to death. The selection of APT reuse time for ICH patients should be cautious because prematurely resuming antiplatelet therapy may potentially increase ICH recurrence risk, whereas unnecessarily delaying the restart of antiplatelet therapy may significantly increase the patient's risk of thromboembolism, and many relative clinical studies are still needed (45, 46). Meanwhile, given the increase in risk, whether platelet function reversal strategies can ameliorate the mortality associated with pre-ICH APT at an early time would require relatively large trials to demonstrate. More medical attention should be given to ICH patients with prior APT use.

We found statistical evidence of heterogeneity in both univariate analyses and multivariable adjusted analyses, and our

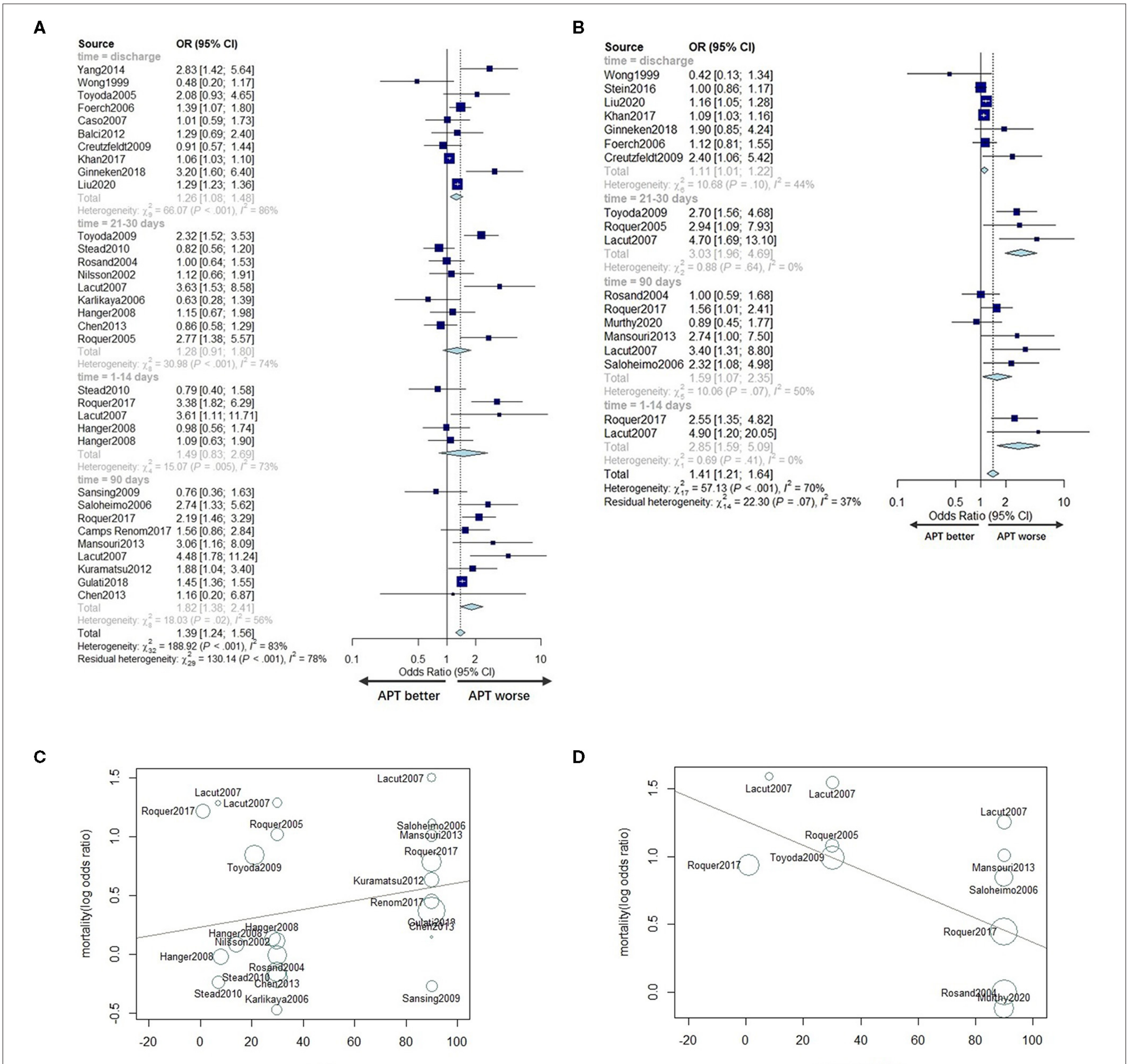

FIGURE 4 | Subgroup analyses according to assessment time in univariate analyses **(A)** and adjusted analyses **(B)**. Meta-regression for the assessment time for univariate analyses **(C)** and multivariable adjusted analyses **(D)**.

univariate meta-regression analysis showed that the difference in study range, study types and continents could be the primary reasons for heterogeneity—different patients exhibit differential drug sensitivities, and different regions have different drug preferences (3). Drug sensitivity, different types of APT (9, 47), dual or triple APT use (8), and the duration or dosage of APT could influence the outcome of ICH (8, 39). In addition, the heterogeneity of the adjusted OR could also be ascribed to the different adjusted factors in the multivariate analysis of each study. Finally, the inherent biases and differences in the designs of the observational studies lead to an increased risk of heterogeneity.

Secondary Outcomes

In addition to mortality, functional outcome is also a hot topic in research. We found that prior APT did not play an unfavorable role in the prognosis of ICH, and the result was consistent with that of Thompson et al. (4).

Moreover, we found that prior APT was not associated with early hematoma growth (HG). All this suggests that hematoma

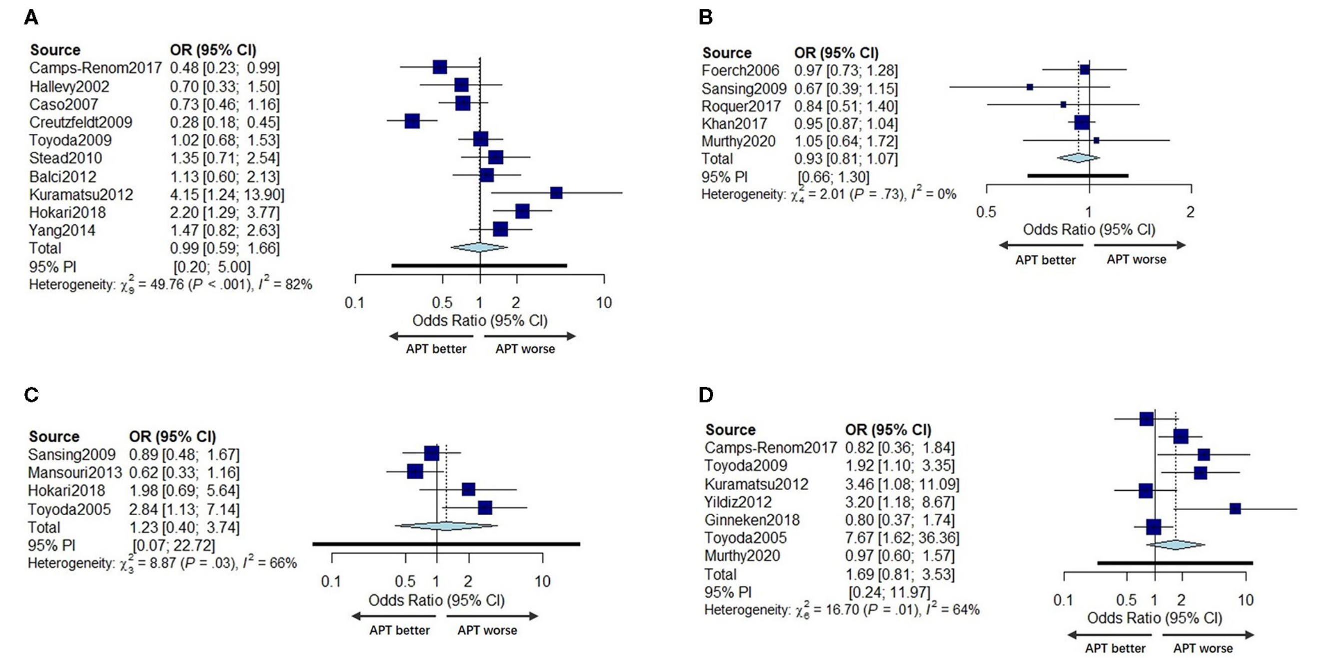

FIGURE 5 | Forest plot of univariate OR **(A)** and multivariable adjusted OR **(B)** for poor function outcome in prior APT users compared to non-users. Forest plot comparing hematoma growth for APT vs. no APT in univariate analyses **(C)** and in multivariable adjusted analyses **(D)**.

TABLE 4 | Egger's test of studies.

Analysis	Egger's test (*p*-value)	Trim-and-fill estimate pooled-changes (95% CI)
Univariate analyses for mortality	0.0398*	−0.17 (−0.15; −0.19)
Multivariable analyses for mortality	0.006*	−0.20 (−0.19; −0.22)
Univariate analyses for Poor function outcome	0.427	-
Multivariable analyses for Poor function outcome	0.449	-
Univariate analyses for hematoma growth	0.286	-
Multivariable analyses for hematoma growth	0.386	-

*$p < 0.05$.

growth may not be a possible mechanism by which prior APT causes higher mortality in ICH patients, which contradicts the results reported by Camps-Renom (11). One possible explanation regarding the discrepancy among studies is that we included more studies with larger sample sizes and separated unadjusted ORs and adjusted ORs for statistical analysis. In addition, another possible explanation may be that platelet activity is not measured directly but inferred from the medical history (11, 13). There may be a threshold effect where the reduction in platelet activity must be substantial enough to influence the outcome (8). Therefore, in the future, further research can conduct more in-depth exploration by directly detecting the platelet activity of patients.

Sensitivity Analysis and Publication Bias

Sensitivity analysis indicated that the results of this study were reliable. However, it should be admitted that the quality of the included studies was indeed at a medium level, which is related to the fact that all studies were non-RCT observational studies. Fifty-seven percent of the included studies were retrospective studies, and some studies were secondary studies. The quality of evidence was downgraded mainly by the retrospective design of the studies. Statistical analysis of patients' prior APT use was accompanied by an inevitable recall bias. Furthermore, due to too many related confounding factors, it is difficult to obtain comprehensive statistics in the studies.

Notably, publication bias indeed exists in the analysis of mortality in studies with adjusted ORs. The results of the trim-and-fill analysis showed that there were no significant changes in the estimate of the combined effect size. Publication bias had little effect on the results, and the results were robust.

Several studies compared the effects of different types of antiplatelet agents on the outcomes in ICH patients. However, the results were not appropriate to be pooled due to the significant heterogeneity. Toyoda et al. (32) compared the effects of aspirin, other single APT use, and dual APT use and found that aspirin use was associated with more 30-day mortality and hematoma enlargement; Liu et al. (9) compared the effects of cyclooxygenase inhibitor (COX-I), adenosine diphosphate receptor inhibitor

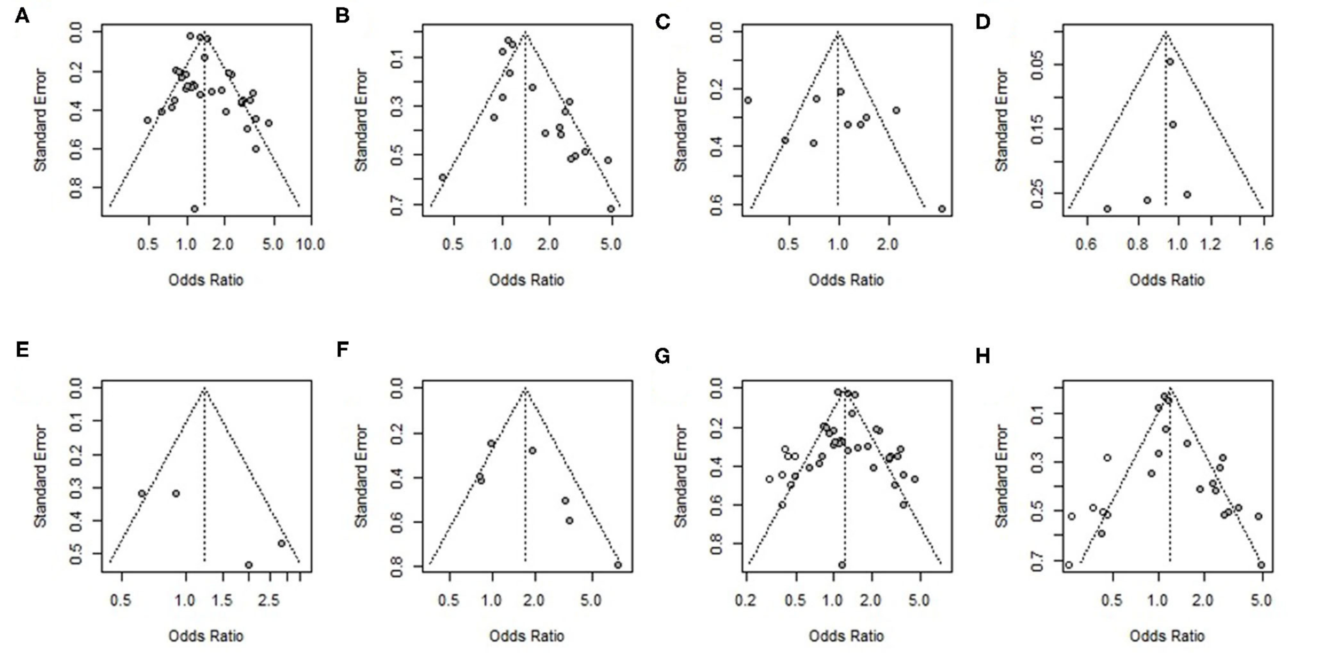

FIGURE 6 | Funnel plot of **(A)** univariate odds ratios for mortality, **(B)** multivariable adjusted odds ratios for mortality, **(C)** univariate odds ratios for poor function outcome, **(D)** multivariable adjusted odds ratios for poor function outcome, **(E)** univariate odds ratios for hematoma growth (HG), **(F)** multivariable adjusted odds ratios for hematoma growth (HG), **(G)** results of the trim-and-fill analysis of univariate odds ratios for mortality, and **(H)** results of the trim-and-fill analysis of univariate odds ratios for mortality.

(ADP-I), and phosphodiesterase inhibitor (PDE-I) and found that ADP-I and COX-1 are the most likely contributors to the poor outcomes in spontaneous ICH patients. Khan et al. (8) suggested that the previous use of CAPT, but not SAPT, was associated with a higher risk of in-hospital mortality among ICH patients. Further studies are needed to explore the effects of different choice, usage, and dosage of antiplatelet agents on the outcomes in ICH patients.

Limitations

The current study has several limitations. First, all the included studies were non-RCT observational cohort studies. Second, data regarding the choice, usage, and dosage of antiplatelet agents were not appropriate to process correlated analysis due to the significant heterogeneity. Large sample RCTs are needed to evaluate these.

CONCLUSION

Implications for Practice

The present study represents that prior APT was not associated with hematoma expansion or functional outcomes, but there was modestly increased mortality in prior APT patients. Safety concerns should be considered when chronic antiplatelet treatment is planned. Additionally, the finding that higher mortality of prior APT patients was related to the strong influence of prior APT use on early mortality suggested that early-time stage in ICH patients with prior APT is crucial, which needs close monitoring and management.

Implications for Research

Whether it is possible to reduce prior APT mortality in ICH patients by restoring early platelet function requires relatively large trials to demonstrate. In addition, our conclusion negates the correlation between prior APT and hematoma expansion; therefore, whether prior APT use could be an independent predictor of early hematoma growth (HG) still needs further exploration. Further research can conduct more in-depth exploration by directly detecting the platelet activity of patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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